



STIC Search Report

Biotech-Chem Library

STIC Database Tracking Number: 154076

TO: Rei-Tsang Shiao
Location: 5a10 / 5c18
Wednesday, May 25, 2005
Art Unit: 1626
Phone: 571-272-0707
Serial Number: 10 / 673487

From: Jan Delaval
Location: Biotech-Chem Library
Remsen 1a51
Phone: 571-272-2504
jan.delaval@uspto.gov

Search Notes

Jan Delaware
for search

Access DB# 154076

SEARCH REQUEST FORM

Scientific and Technical Information Center

Requester's Full Name: Robert (Reis) Shio Examiner #: 7952 Date: 5/20/95
Art Unit: 1626 Phone Number: 2-0707 Serial Number: 10/673, 987
Mail Box and Bldg/Room Location: 5A10/5C10 Results Format Preferred (circle): PAPER DISK E-MAIL

If more than one search is submitted, please prioritize searches in order of need.

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of invention: Just for indexing repts potential
Inventors (please provide full names): Matsuo et al

Earliest Priority Filing Date:

For Sequence Searches Only Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.

I search method of use of organic dye
having formula 14, 15, 16, 17. (see claim 1)

II search methods of use of photostimulation
in the optic nerve, using organic dye,
or cpd formula 14, 15, 16, 17.

STAFF USE ONLY

Searcher: Sam
Searcher Phone #: 22504
Searcher Location:
Date Searcher Picked Up: 5/25/05
Date Completed: 5/25/05
Searcher Pre-Review Time:
Clinical Prep Time: 20
On-line Time: + 30

Type of Search

NA Sequence (H)
AA Sequence (H)
Structure (H) ☒
Bibliographic
Litigation
Fulltext
Patent Family
Other

Vendors and cost where applicable

STN
Dialog
Questel/Orbit
DrLink
Lexis/Nexis
Sequence Systems
WWW/Internet
Other (specify)

=> fil reg

FILE 'REGISTRY' ENTERED AT 16:04:55 ON 25 MAY 2005
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Property values tagged with IC are from the ZIC/VINITI data file
provided by InfoChem.

STRUCTURE FILE UPDATES: 24 MAY 2005 HIGHEST RN 851066-92-7
DICTIONARY FILE UPDATES: 24 MAY 2005 HIGHEST RN 851066-92-7

New CAS Information Use Policies, enter HELP USAGETERMS for details.

TSCA INFORMATION NOW CURRENT THROUGH JANUARY 18, 2005

Please note that search-term pricing does apply when
conducting SmartSELECT searches.

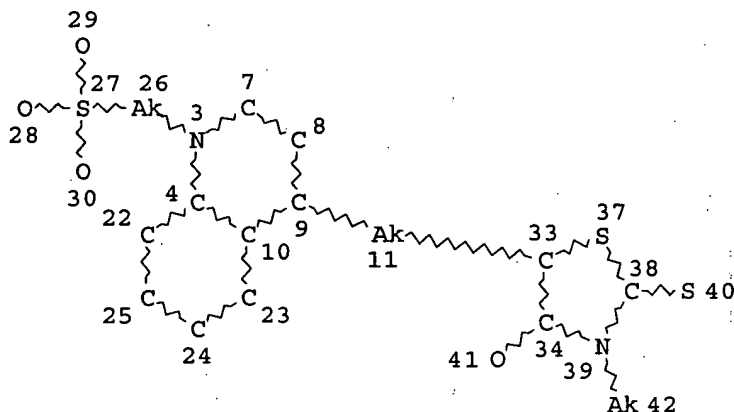
*
* The CA roles and document type information have been removed from *
* the IDE default display format and the ED field has been added, *
* effective March 20, 2005. A new display format, IDERL, is now *
* available and contains the CA role and document type information. *
*

Crossover limits have been increased. See HELP CROSSOVER for details.

Experimental and calculated property data are now available. For more
information enter HELP PROP at an arrow prompt in the file or refer
to the file summary sheet on the web at:
<http://www.cas.org/ONLINE/DBSS/registryss.html>

=> d sta que 127

L21 STR



NODE ATTRIBUTES:
DEFAULT MLEVEL IS ATOM
DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
RING(S) ARE ISOLATED OR EMBEDDED

NUMBER OF NODES IS 24

STEREO ATTRIBUTES: NONE

L27 30 SEA FILE=REGISTRY SSS FUL L21

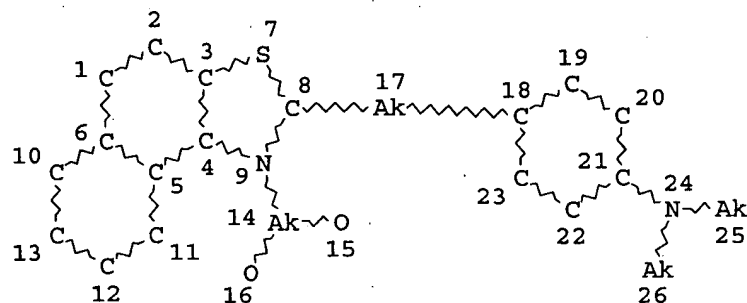
100.0% PROCESSED 2477 ITERATIONS

30 ANSWERS

SEARCH TIME: 00.00.01

=> d sta que l28

L23 STR



NODE ATTRIBUTES:

DEFAULT MLEVEL IS ATOM

DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:

RING(S) ARE ISOLATED OR EMBEDDED

NUMBER OF NODES IS 26

STEREO ATTRIBUTES: NONE

L28 0 SEA FILE=REGISTRY SSS FUL L23

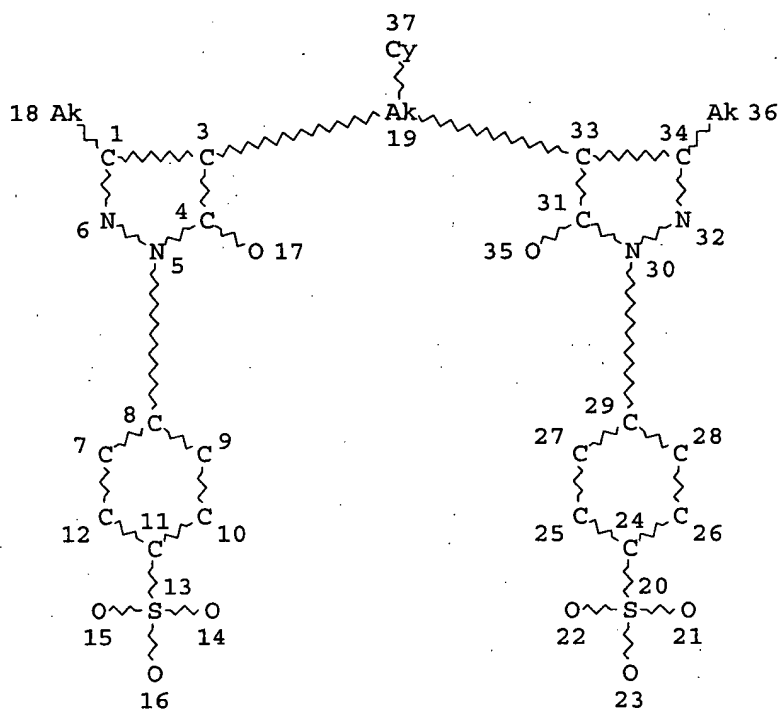
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0 ANSWERS

SEARCH TIME: 00.00.03

=> d sta que l29

L25 STR



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 DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
 RING(S) ARE ISOLATED OR EMBEDDED
 NUMBER OF NODES IS 36

STEREO ATTRIBUTES: NONE
 L29 25 SEA FILE=REGISTRY SSS FUL L25

100.0% PROCESSED 2079 ITERATIONS
 SEARCH TIME: 00.00.01

25 ANSWERS

=> d his

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 SET COST OFF

FILE 'HCAPLUS' ENTERED AT 15:19:35 ON 25 MAY 2005

L1	1 S US20040062713/PN OR (US2003-673487# OR JP2002-285784)/AP, PRN
	E MATSUO T/AU
L2	465 S E3, E4
	E MATSUO TOSH/AU
L3	41 S E7
	E TOSHIHIKO/AU
L4	1 S E3
	E MATSUO/AU
	E KAN OH/AU
L5	1 S E5

jan delaval - 25 may 2005

E KAN O/AU
 E KANO/AU
 E KANOH/AU
 L6 8 S E125
 E YASUFUMI/AU
 E SUGA S/AU
 L7 379 S E3,E4,E8
 E SADAHARU S/AU
 L8 55065 S (KABUSHIKI? OR KAISHA? OR HAYASHIBARA? OR SEIBUTSU? OR KAGAKU
 L9 1 S L1 AND L2-L8
 E POLYMETHIN
 L10 2797 S E3,E5
 E METHIN
 L11 12 S POLY()E3,E20
 L12 2808 S L10,L11
 L13 9 S L1-L8 AND L12
 L14 1 S L13 AND OPTIC?(L)NERV?
 L15 1 S L9,L14
 L16 8 S L13 NOT L15
 SEL RN L16

FILE 'REGISTRY' ENTERED AT 15:29:53 ON 25 MAY 2005

L17 91 S E1-E91
 L18 0 S NCSC2-C6-C6/ES AND C6/ES AND 4/NR AND L17
 L19 0 S NC5-C6/ES AND NCSC2/ES AND 3/NR AND L17
 L20 0 S C6/ES AND N2C3/ES AND 5/NR AND L17
 L21 STR
 L22 3 S L21
 L23 STR
 L24 0 S L23
 L25 STR
 L26 1 S L25
 L27 30 S L21 FUL
 SAV TEMP L27 SHIAO673/A
 L28 0 S L23 FUL
 SAV TEMP L28 SHIAO673A/A
 L29 25 S L25 FUL
 SAV L29 SHIAO673B/A TEMP
 L30 2 S L27 AND C24H28N2O4S3
 L31 3 S L29 AND C31H26N4O8S2
 L32 1596 S NCSC2-C6-C6/ES AND C6/ES AND 4/NR
 L33 154 S L32 AND 2/N AND 2/O AND 1/S
 L34 4 S L33 AND BR/ELS
 L35 1 S L34 AND C23H21N2O2S
 L36 10 S L33 AND IUM NOT L34
 L37 140 S L33 NOT L34-L36
 L38 1 S L36 AND C23H21N2O2S
 L39 5 S L30,L31
 SAV L39 SHIAO673C/A TEMP

FILE 'HCAOLD' ENTERED AT 15:49:23 ON 25 MAY 2005

L40 0 S L39

FILE 'HCAPLUS' ENTERED AT 15:49:27 ON 25 MAY 2005

L41 9 S L39
 L42 19 S NK2761 ORNK3041 OR NK() (2761 OR 3041) OR RH155 OR RH 155
 L43 20 S L41,L42
 L44 1 S L43 AND L1-L8
 SEL RN

FILE 'REGISTRY' ENTERED AT 15:50:09 ON 25 MAY 2005

L45 8 S E92-E99
L46 7 S L45 NOT CA/ELS
SEL RN 1 2 3 4
L47 4 S E100-E103

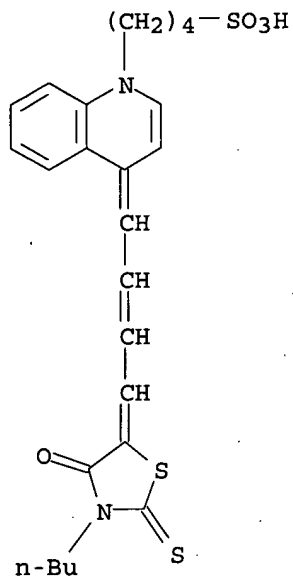
FILE 'HCAPLUS' ENTERED AT 15:52:33 ON 25 MAY 2005

L48 10 S L47
L49 10 S NK5962 OR NK3630 OR NK() (5962 OR 3630) OR RH482 OR RH 482
L50 27 S L43,L48,L49
L51 2 S L1-L8 AND L50
L52 3 S L15,L51
L53 2 S L52 NOT SEMICONDUCTOR
L54 27 S L50 AND (PD<=20030930 OR PRD<=20030930 OR AD<=20030930)
L55 9 S L54 AND OPTIC?(L)NERV?
L56 1 S L54 AND (RETINA OR RETINAL OR RETINO?)
L57 1 S L54 AND EYE
E EYE/CT
L58 1 S L54 AND E3+OLD,NT,PRT,RT
L59 1 S L54 AND E3-E151
L60 0 S L54 AND E179
L61 0 S L54 AND E183
L62 0 S L54 AND E214
L63 1 S L54 AND E215+OLD,NT,PFT,RT
L64 0 S L54 AND E215-E307
L65 0 S L54 AND E307+OLD,NT,PFT,RT
L66 0 S L54 AND E307-E322
L67 0 S L54 AND E323-E336
L68 0 S L54 AND E323+OLD,NT,PFT,RT
L69 0 S L54 AND E325+OLD,NT,PFT,RT
L70 0 S L54 AND E337-E341
E RETINA/CT
E S L52 AND E3
E RETINA/CT
L71 0 S L54 AND E3
E E3+ALL
L72 1 S L54 AND E2
L73 10 S L55-L72
L74 11 S L53,L73
L75 17 S L54 NOT L74
L76 8 S L74 AND L43
L77 3 S L74 NOT L76

FILE 'REGISTRY' ENTERED AT 16:04:55 ON 25 MAY 2005

=> d ide can tot l39

L39 ANSWER 1 OF 5 REGISTRY COPYRIGHT 2005 ACS on STN
RN 693210-77-4 REGISTRY
ED Entered STN: 14 Jun 2004
CN 1(4H)-Quinolinebutanesulfonic acid, 4-[4-(3-butyl-4-oxo-2-thioxo-5-thiazolidinylidene)-2-butenylidene]- (9CI) (CA INDEX NAME)
FS 3D CONCORD
MF C24 H28 N2 O4 S3
CI COM
SR CA



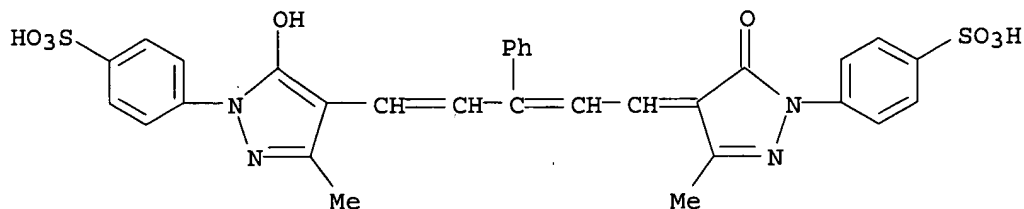
PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

L39 ANSWER 2 OF 5 REGISTRY COPYRIGHT 2005 ACS on STN
 RN 254451-42-8 REGISTRY
 ED Entered STN: 02 Feb 2000
 CN Benzenesulfonic acid, 4-[4,5-dihydro-4-[5-[5-hydroxy-3-methyl-1-(4-sulfophenyl)-1H-pyrazol-4-yl]-3-phenyl-2,4-pentadienylidene]-3-methyl-5-oxo-1H-pyrazol-1-yl]-, compd. with N,N-diethylethanamine (1:3) (9CI) (CA INDEX NAME)
 MF C31 H26 N4 O8 S2 . 3 C6 H15 N
 SR CAS Client Services
 LC STN Files: CHEMCATS, CSCHEM

CM 1

CRN 254451-41-7

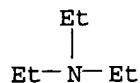
CMF C31 H26 N4 O8 S2



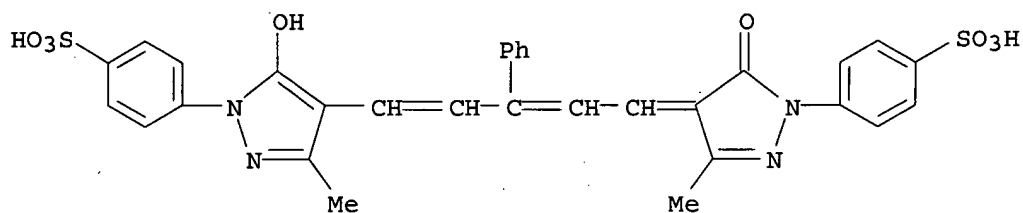
CM 2

CRN 121-44-8

CMF C6 H15 N

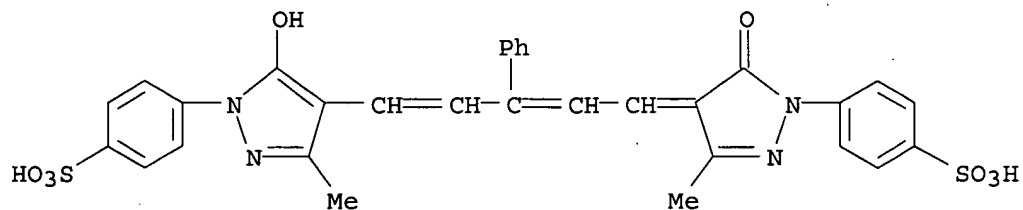


L39 ANSWER 3 OF 5 REGISTRY COPYRIGHT 2005 ACS on STN
 RN 254451-41-7 REGISTRY
 ED Entered STN: 02 Feb 2000
 CN Benzenesulfonic acid, 4-[4,5-dihydro-4-[5-[5-hydroxy-3-methyl-1-(4-sulfophenyl)-1H-pyrazol-4-yl]-3-phenyl-2,4-pentadienylidene]-3-methyl-5-oxo-1H-pyrazol-1-yl]- (9CI) (CA INDEX NAME)
 MF C31 H26 N4 O8 S2
 CI COM
 SR CAS Client Services



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

L39 ANSWER 4 OF 5 REGISTRY COPYRIGHT 2005 ACS on STN
 RN 135806-37-0 REGISTRY
 ED Entered STN: 30 Aug 1991
 CN Benzenesulfonic acid, 4-[4,5-dihydro-4-[5-[5-hydroxy-3-methyl-1-(4-sulfophenyl)-1H-pyrazol-4-yl]-3-phenyl-2,4-pentadienylidene]-3-methyl-5-oxo-1H-pyrazol-1-yl]-, trisodium salt (9CI) (CA INDEX NAME)
 OTHER NAMES:
 CN NK 3041
 CN RH 155
 MF C31 H26 N4 O8 S2 . 3 Na
 SR CA
 LC STN Files: BIOSIS, CA, CAPLUS, TOXCENTER, USPATFULL
 CRN (254451-41-7)



● 3 Na

8 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
8 REFERENCES IN FILE CAPLUS (1907 TO DATE)

REFERENCE 1: 141:240
REFERENCE 2: 140:387829
REFERENCE 3: 136:196477
REFERENCE 4: 133:330852
REFERENCE 5: 132:290585
REFERENCE 6: 132:90253
REFERENCE 7: 127:314237
REFERENCE 8: 115:125933

L39 ANSWER 5 OF 5 REGISTRY COPYRIGHT 2005 ACS on STN

RN 79953-79-0 REGISTRY

ED Entered STN: 16 Nov 1984

CN 1(4H)-Quinolinebutanesulfonic acid, 4-[4-(3-butyl-4-oxo-2-thioxo-5-thiazolidinyldene)-2-butenylidene]-, sodium salt (9CI) (CA INDEX NAME)

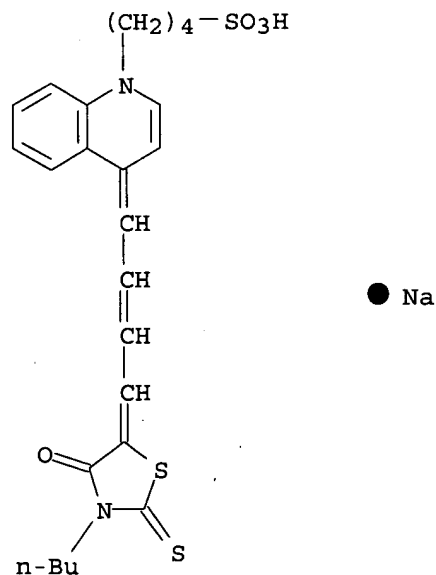
OTHER NAMES:

CN NK 2761

MF C24 H28 N2 O4 S3 . Na

LC STN Files: BIOSIS, CA, CAPLUS, EMBASE, MEDLINE, TOXCENTER

CRN (693210-77-4)



4 REFERENCES IN FILE CA (1907 TO DATE)
4 REFERENCES IN FILE CAPLUS (1907 TO DATE)

REFERENCE 1: 141:240

REFERENCE 2: 136:196477

REFERENCE 3: 132:90253

REFERENCE 4: 118:187199

=> d ide can tot.l47

L47 ANSWER 1 OF 4 REGISTRY COPYRIGHT 2005 ACS on STN

RN 577975-80-5 REGISTRY

ED Entered STN: 03 Sep 2003

CN Benzothiazolium, 3-(carboxymethyl)-2-[2-[4-(dibutylamino)phenyl]ethenyl]-, bromide (9CI) (CA INDEX NAME)

OTHER NAMES:

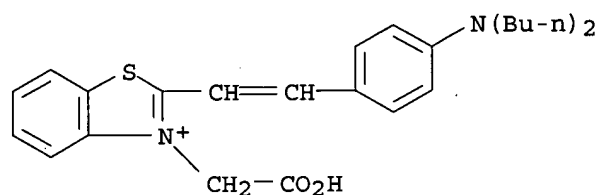
CN NK 5962

MF C25 H31 N2 O2 S . Br

SR CA

LC STN Files: CA, CAPLUS

CRN (732982-26-2)



● Br⁻

2 REFERENCES IN FILE CA (1907 TO DATE)

2 REFERENCES IN FILE CAPLUS (1907 TO DATE)

REFERENCE 1: 141:240

REFERENCE 2: 139:182865

L47 ANSWER 2 OF 4 REGISTRY COPYRIGHT 2005 ACS on STN

RN 254729-07-2 REGISTRY

ED Entered STN: 03 Feb 2000

CN Benzenesulfonic acid, 4-[4,5-dihydro-4-[5-[5-hydroxy-3-propyl-1-(4-sulfophenyl)-1H-pyrazol-4-yl]-3-phenyl-2,4-pentadienylydene]-5-oxo-3-propyl-1H-pyrazol-1-yl]-, trisodium salt (9CI) (CA INDEX NAME)

OTHER NAMES:

CN NK 3630

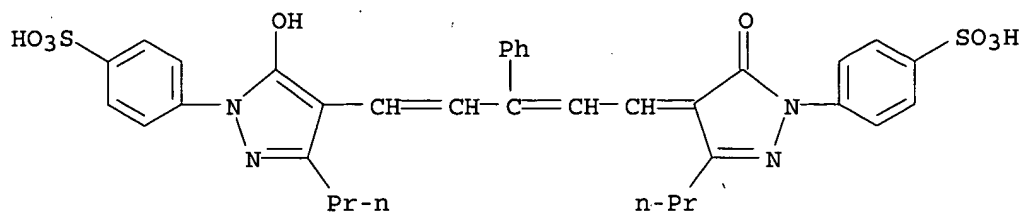
CN RH 482

MF C35 H34 N4 O8 S2 . 3 Na

SR CA

LC STN Files: BIOSIS, CA, CAPLUS, TOXCENTER

CRN (781601-37-4)



● 3 Na

3 REFERENCES IN FILE CA (1907 TO DATE)
3 REFERENCES IN FILE CAPLUS (1907 TO DATE)

REFERENCE 1: 141:240

REFERENCE 2: 140:387829

REFERENCE 3: 132:90253

L47 ANSWER 3 OF 4 REGISTRY COPYRIGHT 2005 ACS on STN

RN 135806-37-0 REGISTRY

ED Entered STN: 30 Aug 1991

CN Benzenesulfonic acid, 4-[4,5-dihydro-4-[5-[5-hydroxy-3-methyl-1-(4-sulfophenyl)-1H-pyrazol-4-yl]-3-phenyl-2,4-pentadienylydene]-3-methyl-5-oxo-1H-pyrazol-1-yl]-, trisodium salt (9CI) (CA INDEX NAME)

OTHER NAMES:

CN NK 3041

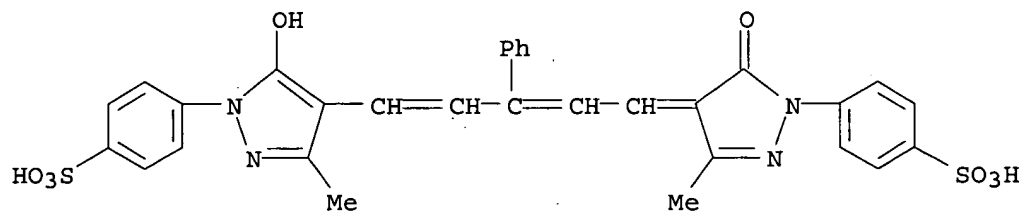
CN RH 155

MF C31 H26 N4 O8 S2 . 3 Na

SR CA

LC STN Files: BIOSIS, CA, CAPLUS, TOXCENTER, USPATFULL

CRN (254451-41-7)



● 3 Na

8 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
8 REFERENCES IN FILE CAPLUS (1907 TO DATE)

REFERENCE 1: 141:240

REFERENCE 2: 140:387829

REFERENCE 3: 136:196477

REFERENCE 4: 133:330852

REFERENCE 5: 132:290585

REFERENCE 6: 132:90253

REFERENCE 7: 127:314237

REFERENCE 8: 115:125933

L47 ANSWER 4 OF 4 REGISTRY COPYRIGHT 2005 ACS on STN

RN 79953-79-0 REGISTRY

ED Entered STN: 16 Nov 1984

CN 1(4H)-Quinolinebutanesulfonic acid, 4-[4-(3-butyl-4-oxo-2-thioxo-5-thiazolidinylidene)-2-butenylidene]-, sodium salt (9CI) (CA INDEX NAME)

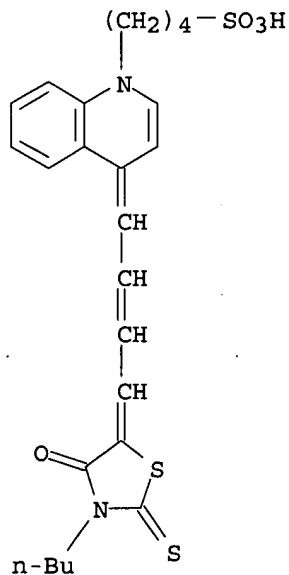
OTHER NAMES:

CN NK 2761

MF C24 H28 N2 O4 S3 . Na

LC STN Files: BIOSIS, CA, CAPLUS, EMBASE, MEDLINE, TOXCENTER

CRN (693210-77-4)



4 REFERENCES IN FILE CA (1907 TO DATE)

4 REFERENCES IN FILE CAPLUS (1907 TO DATE)

REFERENCE 1: 141:240

REFERENCE 2: 136:196477

REFERENCE 3: 132:90253

REFERENCE 4: 118:187199

=> fil hcaplus
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FILE COVERS 1907 - 25 May 2005 VOL 142 ISS 22
FILE LAST UPDATED: 24 May 2005 (20050524/ED)

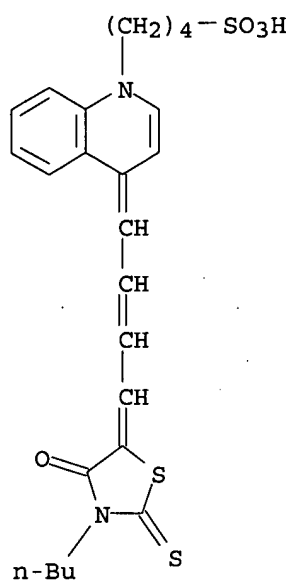
New CAS Information Use Policies, enter HELP USAGETERMS for details.

This file contains CAS Registry Numbers for easy and accurate substance identification.

=> => d all hitstr tot 176

L76 ANSWER 1 OF 8 HCAPLUS COPYRIGHT 2005 ACS on STN
AN 2003:920680 HCAPLUS
DN 141:240
ED Entered STN: 25 Nov 2003
TI A simple method for screening photoelectric dyes towards their use for
retinal prostheses
AU Matsuo, Toshihiko
CS Department of Ophthalmology, Okayama University Graduate School of
Medicine and Dentistry, Okayama, 700-8558, Japan
SO Acta Medica Okayama (2003), 57(5), 257-260
CODEN: AMOKAG; ISSN: 0386-300X
PB Okayama University Medical School
DT Journal
LA English
CC 1-1 (Pharmacology)
AB Photoelec. dyes absorb light and convert photon energy to elec.
potentials. To test whether these dyes could be used for retinal
prostheses, a simple in vitro screening system was developed.
Retinal neurons were cultured from the eyes of chick
embryos at the 10-day embryonic stage, at which time no retinal
photoreceptor cells have yet developed. Intracellular calcium elevation
was observed with Fluo-4 in cultured retinal neurons before and
after photoelec. dye was applied at varying concns. to the culture medium.
Five of 7 photoelec. dyes tested in this in vitro system induced
intracellular calcium elevation in cultured chick retinal
neurons. The intracellular calcium elevation generated by the 5
photoelec. dyes was blocked by extracellular calcium depletion in the case
of all 5 dyes, and, except for one dye, by the presence of voltage-gated
calcium channel blockers. The photoelec. dyes absorbed light under an
inverted microscope and stimulated retinal neurons. This simple
in vitro system allows the screening of photoelec. dyes which can be used
for retinal prostheses.

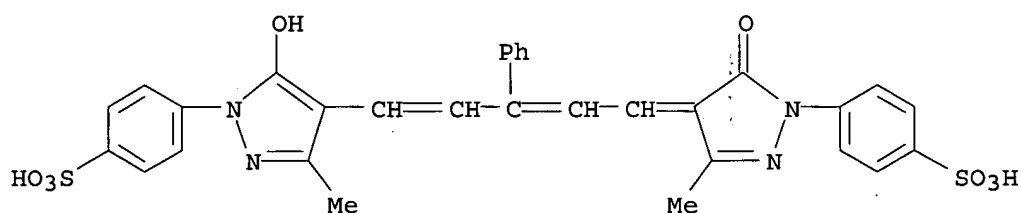
ST photoelec dye screening **retina** neuron prosthetic
IT Prosthetic materials and Prosthetics
(implants, **retinal**; simple method for screening photoelec.
dyes toward their use for **retinal** prostheses)
IT Dyes
(photoelec.; simple method for screening photoelec. dyes toward their
use for **retinal** prostheses)
IT **Eye**
(**retina**, **neural**; simple method for screening
photoelec. dyes toward their use for **retinal** prostheses)
IT Drug screening
Light
(simple method for screening photoelec. dyes toward their use for
retinal prostheses)
IT Calcium channel
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(voltage-gated; simple method for screening photoelec. dyes toward
their use for **retinal** prostheses)
IT 7440-70-2, Calcium, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(intracellular; simple method for screening photoelec. dyes toward
their use for **retinal** prostheses)
IT 25962-03-2, NK 2045 28782-33-4, NK 5078 33628-03-4, NK 1952
79953-79-0, NK 2761 135806-37-0,
NK 3041 254729-07-2, NK 3630
577975-80-5, NK 5962
RL: PAC (Pharmacological activity); THU (Therapeutic use); BIOL
(Biological study); USES (Uses)
(simple method for screening photoelec. dyes toward their use for
retinal prostheses)
RE.CNT 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
(1) Adler, R; Dev Neurosci 1982, V5, P27 MEDLINE
(2) Humayun, M; Arch Ophthalmol 1996, V114, P40 MEDLINE
(3) Humayun, M; Trans Am Ophthalmol Soc 2001, V99, P271 MEDLINE
(4) Matsuo, T; Acta Med Okayama 1997, V51, P251 HCAPLUS
(5) Matsuo, T; Br J Ophthalmol 1996, V80, P561 MEDLINE
(6) Namba, M; Ophthalmic Res 2001, V33, P163 HCAPLUS
(7) Peyman, G; Ophthalmic Surg Lasers 1998, V29, P234 MEDLINE
(8) Zrenner, E; Science 2002, V295, P1022 HCAPLUS
IT 79953-79-0, NK 2761 135806-37-0,
NK 3041 254729-07-2, NK 3630
577975-80-5, NK 5962
RL: PAC (Pharmacological activity); THU (Therapeutic use); BIOL
(Biological study); USES (Uses)
(simple method for screening photoelec. dyes toward their use for
retinal prostheses)
RN 79953-79-0 HCAPLUS
CN 1(4H)-Quinolinebutanesulfonic acid, 4-[4-(3-butyl-4-oxo-2-thioxo-5-
thiazolidinylidene)-2-butenylidene]-, sodium salt (9CI) (CA INDEX NAME)



● Na

RN 135806-37-0 HCAPLUS

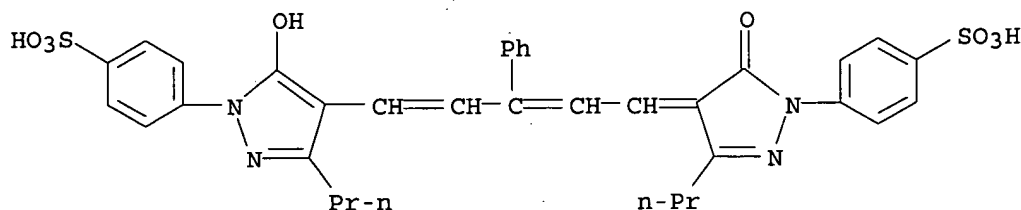
CN Benzenesulfonic acid, 4-[4,5-dihydro-4-[5-[5-hydroxy-3-methyl-1-(4-sulfophenyl)-1H-pyrazol-4-yl]-3-phenyl-2,4-pentadienylidene]-3-methyl-5-oxo-1H-pyrazol-1-yl]-, trisodium salt (9CI) (CA INDEX NAME)



● 3 Na

RN 254729-07-2 HCAPLUS

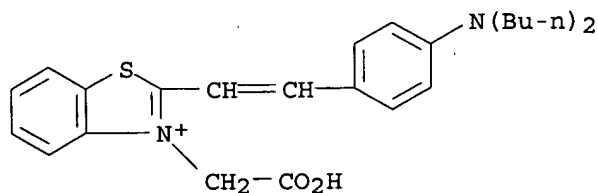
CN Benzenesulfonic acid, 4-[4,5-dihydro-4-[5-[5-hydroxy-3-propyl-1-(4-sulfophenyl)-1H-pyrazol-4-yl]-3-phenyl-2,4-pentadienylidene]-5-oxo-3-propyl-1H-pyrazol-1-yl]-, trisodium salt (9CI) (CA INDEX NAME)



● 3 Na

RN 577975-80-5 HCAPLUS

CN Benzothiazolium, 3-(carboxymethyl)-2-[2-[4-(dibutylamino)phenyl]ethenyl]-, bromide (9CI) (CA INDEX NAME)

● Br⁻

L76 ANSWER 2 OF 8 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 2001:446421 HCAPLUS

DN 136:196477

ED Entered STN: 21 Jun 2001

TI Visualization of activity in the nucleus related to the eighth nerve in the brainstem

AU Doi, Tadashi; Asako, Mikiya; Matsumoto-Ono, Ayumi; Kaneko, Toshihiko; Yamashita, Toshio

CS Dep. Otolaryngol., Kansai Med. Univ., Japan

SO Otolology Japan (2001), 11(2), 92-96

CODEN: OTJAEW; ISSN: 0917-2025

PB Nippon Jika Gakkai

DT Journal

LA Japanese

CC 9-16 (Biochemical Methods)

Section cross-reference(s): 12, 13

AB We investigated **optical** imaging of the evoked responses in chick embryo and mouse the cochlear and vestibular nucleus in the brainstem slices by elec. stimulation, of the vestibulocochlear **nerve** using a multiple-site **optical** recording system and an absorption voltage-sensitive dye, NK2761 and RH155. The spatiotemporal patterns of excitatory propagation in the cochlear and vestibular nucleus were shown with **optical** imaging. These **optical** signals were wavelength dependent and consisted of two components spike-like fast signal and long-lasting slow signal. All responses were abolished by tetrodotoxin. The slow signals were eliminated under

bath-applied Ca²⁺-free solution The effect of Ca²⁺-free was reversible. Synaptic fatigue was observed when repetitive stimulation was applied to the vestibulocochlear **nerve**. These results suggest that the neural activities through the sodium channels gave rise to the fast responses and the slow signals corresponded to a postsynaptic potential. The present study indicated the feasibility of **optical** recording for revealing visually the synaptic transmission in the vestibular nucleus and cochlear nucleus in the brainstem with high spatiotemporal resolution activity nucleus eighth nerve brainstem

ST
IT Dyes
 (Absorption voltage-sensitive; visualization of activity in nucleus related to eighth nerve in brainstem)

IT Nerve
 (Vestibulocochlear; visualization of activity in nucleus related to eighth nerve in brainstem)

IT Brain
 (cochlear nucleus; visualization of activity in nucleus related to eighth nerve in brainstem)

IT Synapse
 (fatigue; visualization of activity in nucleus related to eighth nerve in brainstem)

IT Synapse.
 (postsynapse; visualization of activity in nucleus related to eighth nerve in brainstem)

IT Brain
 (stem; visualization of activity in nucleus related to eighth nerve in brainstem)

IT Neurotransmission
 (synaptic; visualization of activity in nucleus related to eighth nerve in brainstem)

IT Nerve
 (toxicity, Vestibulocochlear; visualization of activity in nucleus related to eighth nerve in brainstem)

IT Embryo, animal
Nerve
 (toxicity; visualization of activity in nucleus related to eighth nerve in brainstem)

IT Brain
 (vestibular nucleus; visualization of activity in nucleus related to eighth nerve in brainstem)

IT Electric current
Embryo, animal
Gallus domesticus
Imaging
Mus
Nerve
 Optical recording
Wavelength
 (visualization of activity in nucleus related to eighth **nerve** in brainstem)

IT Sodium channel
RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (visualization of activity in nucleus related to eighth nerve in brainstem)

IT 7440-70-2, Calcium, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
 (visualization of activity in nucleus related to eighth nerve in brainstem)

IT 4368-28-9, Tetrodotoxin 79953-79-0, Nk2761 135806-37-0
 , RH155

RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(visualization of activity in nucleus related to eighth nerve in brainstem)

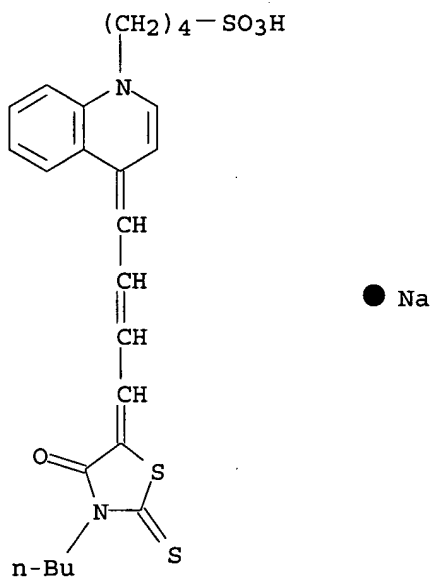
IT 79953-79-0, Nk2761 135806-37-0, RH155

RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(visualization of activity in nucleus related to eighth nerve in brainstem)

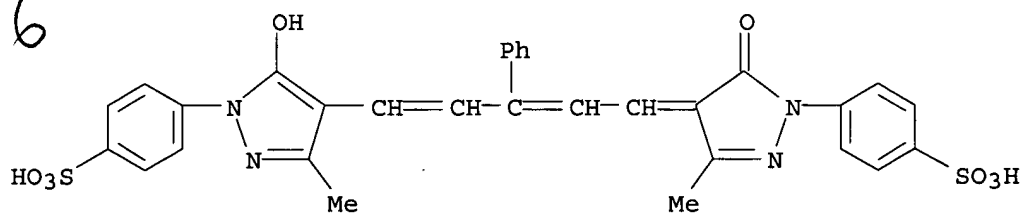
RN 79953-79-0 HCAPLUS

CN 1(4H)-Quinolinebutanesulfonic acid, 4-[4-(3-butyl-4-oxo-2-thioxo-5-thiazolidinylidene)-2-butenylidene]-, sodium salt (9CI) (CA INDEX NAME)



RN 135806-37-0 HCAPLUS

CN Benzenesulfonic acid, 4-[4,5-dihydro-4-[5-[5-hydroxy-3-methyl-1-(4-sulfophenyl)-1H-pyrazol-4-yl]-3-phenyl-2,4-pentadienylidene]-3-methyl-5-oxo-1H-pyrazol-1-yl]-, trisodium salt (9CI) (CA INDEX NAME)



✓ L76 ANSWER 3 OF 8 HCAPLUS COPYRIGHT 2005 ACS on STN
AN 2000:871825 HCAPLUS
DN 134:98282

ED Entered STN: 13 Dec 2000
 TI Neuron-independent Ca²⁺ signaling in glial cells of snail's brain
 AU Kojima, S.; Ogawa, H.; Kouuchi, T.; Nidaira, T.; Hosono, T.; Ito, E.
 CS Laboratory of Animal Behavior and Intelligence, Division of Biological
 Sciences, Graduate School of Science, Hokkaido University, Sapporo,
 060-0810, Japan
 SO Neuroscience (Oxford) (2000), 100(4), 893-900
 CODEN: NRSCDN; ISSN: 0306-4522
 PB Elsevier Science Ltd.
 DT Journal
 LA English
 CC 12-6 (Nonmammalian Biochemistry)
 AB To directly monitor the glial activity in the CNS of the pond snail,
 Lymnaea stagnalis, we **optically** measured the elec. responses in
 the cerebral ganglion and median lip **nerve** to elec. stimulation
 of the distal end of the median lip **nerve**. Using a
 voltage-sensitive dye, **RH155**, we detected a composite
 depolarizing response in the cerebral ganglion, which consisted of a fast
 transient depolarizing response corresponding to a compound action potential
 and a slow depolarizing response. The slow depolarizing response was
 observed more clearly in an isolated median lip **nerve** and also
 detected by extracellular recording. In the median lip **nerve**
 preparation, the slow depolarizing response was suppressed by an L-type Ca²⁺
 channel blocker, nifedipine, and was resistant to tetrodotoxin and
 Na⁺-free conditions. Together with the fact that a delay from the compound
 action potential to the slow depolarizing response was not constant, these
 results suggested that the slow depolarizing response was not a
 postsynaptic response. Because the signals of the action potentials
 appeared on the saturated slow depolarizing responses during repetitive
 stimulation, the slow depolarizing response was suggested to originate
 from glial cells. The contribution of the L-type Ca²⁺ current to the slow
 depolarizing response was confirmed by **optical** recording in the
 presence of Ba²⁺ and also supported by intracellular Ca²⁺ measurement.
 Our results suggested that elec. stimulation directly triggers glial Ca²⁺
 entry through L-type Ca²⁺ channels, providing evidence for the generation
 of glial depolarization independent of neuronal activity in invertebrates.
 ST calcium signaling glial cell snail brain; Lymnaea brain glial cell calcium
 signaling
 IT Calcium channel
 RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL
 (Biological study); PROC (Process)
 (L-type; neuron-independent Ca²⁺ signaling in glial cells of snail's
 brain)
 IT Electric potential
 (biol., action; neuron-independent Ca²⁺ signaling in glial cells of
 snail's brain)
 IT Nervous system
 (central; neuron-independent Ca²⁺ signaling in glial cells of snail's
 brain)
 IT Ganglion
 (cerebral; neuron-independent Ca²⁺ signaling in glial cells of snail's
 brain)
 IT Biological transport
 (channel-mediated; neuron-independent Ca²⁺ signaling in glial cells of
 snail's brain)
 IT Polarization
 (depolarization, biol.; neuron-independent Ca²⁺ signaling in glial
 cells of snail's brain)
 IT Electric current
 (ionic, biol.; neuron-independent Ca²⁺ signaling in glial cells of

snail's brain)
 IT Brain
 Lymnaea stagnalis
 Nerve
 Neuroglia
 Signal transduction, biological
 (neuron-independent Ca2+ signaling in glial cells of snail's brain)
 IT Synapse
 (postsynapse; neuron-independent Ca2+ signaling in glial cells of
 snail's brain)
 IT 7440-70-2, Calcium, biological studies
 RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL
 (Biological study); PROC (Process)
 (neuron-independent Ca2+ signaling in glial cells of snail's brain)

RE.CNT 48 THERE ARE 48 CITED REFERENCES AVAILABLE FOR THIS RECORD

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L76 ANSWER 4 OF 8 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 2000:619975 HCAPLUS

ED Entered STN: 06 Sep 2000

TI Multiple-site optical recording of mouse brainstem evoked by vestibulocochlear nerve stimulation

AU Yang, S.-M.; Doi, T.; Asako, M.; Matsumoto-Ono, A.; Kaneko, T.; Yamashita, T.

CS Department of Otolaryngology, Kansai Medical University, Osaka, 570-8507, Japan

SO Brain Research (2000), 877(1), 95-100

CODEN: BRREAP; ISSN: 0006-8993

PB Elsevier Science B.V.

DT Journal

LA English

AB We used optical imaging to investigate the mouse cochlear and vestibular nucleus in brainstem slices using a voltage-sensitive dye, RH 155. As a result, the spatiotemporal patterns of excitatory propagation were shown. These optical signals consisted of two components consisting of a spike-like fast signal and a long-lasting slow signal. All responses were abolished by tetrodotoxin. The slow signals were eliminated under a Ca²⁺-free solution. In addition, synaptic fatigue was also observed. The present study indicated the feasibility of optical recording for visually revealing the synaptic transmission in both the vestibular and cochlear nucleus.

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L76 ANSWER 5 OF 8 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1999:742701 HCAPLUS

DN 132:90253

ED Entered STN: 23 Nov 1999

TI Evaluation of voltage-sensitive dyes for long-term recording of neural activity in the hippocampus

AU Momose-Sato, Y.; Sato, K.; Arai, Y.; Yazawa, I.; Mochida, H.; Kamino, K.

CS Department of Physiology, Tokyo Medical and Dental University School of Medicine, Tokyo, 113-8519, Japan

SO Journal of Membrane Biology (1999), 172(2), 145-157

CODEN: JMBBBO; ISSN: 0022-2631

PB Springer-Verlag New York Inc.

DT Journal

LA English

CC 9-5 (Biochemical Methods)

AB We searched for an optimal voltage-sensitive dye for optical measurements of neural activity in the hippocampal slice by evaluating several merocyanine-rhodanine and oxonol dyes. The wavelength dependence (action spectra), pharmacol. effects of staining, signal size, signal-to-noise ratio, and the utility of the dyes for long-term continuous recording were examined for four merocyanine-rhodanine dyes (NK 2761, NK 2776, NK 3224 and NK 3225), which had been reported to be optimal in embryonic nervous systems, and for two oxonol dyes (NK 3630 (RH 482) and NK 3041 (RH 155)), which have been among the most popular potentiometric probes for the hippocampal slice preparation. NK 2761, NK 3224 and NK 3225 provided large signal-to-noise ratios, and proved to be useful for optical recordings lasting several hours. NK 3630 was most suitable for long-term recording, although the signal-to-noise ratio was slightly inferior to that of the merocyanine-rhodanines. Using NK 3630 (RH 482) on the hippocampal slice preparation, we demonstrate here that long-term potentiation can be monitored stably for more than 8 h.

ST voltage dye recording neuron activity hippocampus

IT Embryo, animal

Nerve

Nervous system

Optical recording

Pharmacology

Potentiometry

Spectra

Staining, biological

(evaluation of voltage-sensitive dyes for long-term recording of neural activity in hippocampus)

IT Brain

(hippocampus; evaluation of voltage-sensitive dyes for long-term recording of neural activity in hippocampus)

IT Neurotransmission

(long-term potentiation; evaluation of voltage-sensitive dyes for long-term recording of neural activity in hippocampus)

IT Dyes

(voltage-sensitive; evaluation of voltage-sensitive dyes for long-term recording of neural activity in hippocampus)

IT 254732-33-7, NK 2776

RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(NK 2776; evaluation of voltage-sensitive dyes for long-term recording of neural activity in hippocampus)

IT 135806-37-0, RH 155

RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(NK 3041, RH 155; evaluation of voltage-sensitive dyes for long-term recording of neural activity in hippocampus)

IT 254732-68-8, NK 3224

RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(NK 3224; evaluation of voltage-sensitive dyes for long-term recording of neural activity in hippocampus)

- IT 254732-70-2, NK 3225
 RL: BUU (Biological use, unclassified); BIOL (Biological study); USES
 (Uses)
 (NK 3225; evaluation of voltage-sensitive dyes for long-term recording
 of neural activity in hippocampus)
- IT 254729-07-2, NK 3630
 RL: BUU (Biological use, unclassified); BIOL (Biological study); USES
 (Uses)
 (NK 3630, RH 482; evaluation of
 voltage-sensitive dyes for long-term recording of neural activity in
 hippocampus)
- IT 79953-79-0, Nk2761
 RL: BUU (Biological use, unclassified); BIOL (Biological study); USES
 (Uses)
 (evaluation of voltage-sensitive dyes for long-term recording of neural
 activity in hippocampus)

RE.CNT 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD
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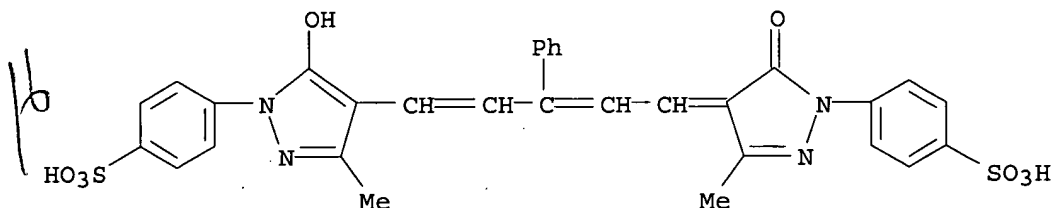
IT 135806-37-0, RH 155

RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(NK 3041, RH 155; evaluation of voltage-sensitive dyes for long-term recording of neural activity in hippocampus)

RN 135806-37-0 HCAPLUS

CN Benzenesulfonic acid, 4-[4,5-dihydro-4-[5-[5-hydroxy-3-methyl-1-(4-sulfophenyl)-1H-pyrazol-4-yl]-3-phenyl-2,4-pentadienyldene]-3-methyl-5-oxo-1H-pyrazol-1-yl]-, trisodium salt (9CI) (CA INDEX NAME)



●3 Na

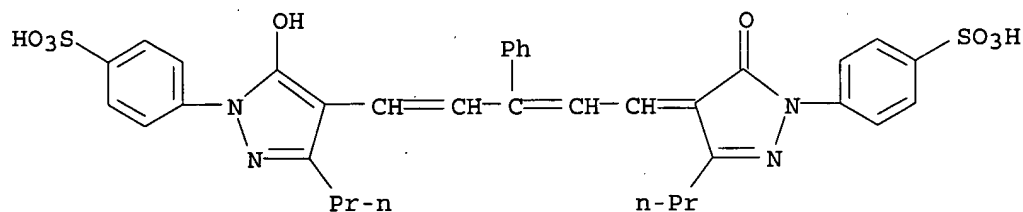
IT 254729-07-2, NK 3630

RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(NK 3630, RH 482; evaluation of voltage-sensitive dyes for long-term recording of neural activity in hippocampus)

RN 254729-07-2 HCAPLUS

CN Benzenesulfonic acid, 4-[4,5-dihydro-4-[5-[5-hydroxy-3-propyl-1-(4-sulfophenyl)-1H-pyrazol-4-yl]-3-phenyl-2,4-pentadienyldene]-5-oxo-3-propyl-1H-pyrazol-1-yl]-, trisodium salt (9CI) (CA INDEX NAME)



●3 Na

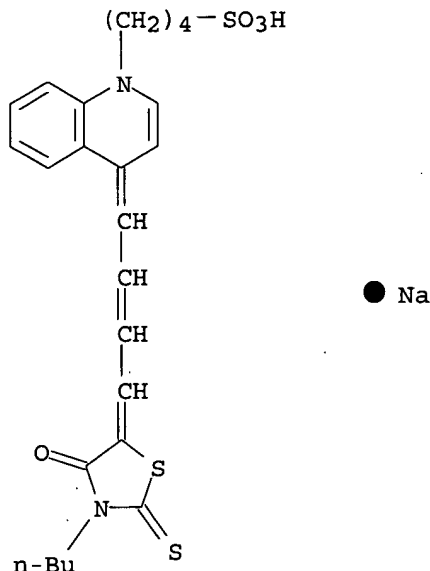
IT 79953-79-0, Nk2761

RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)

(evaluation of voltage-sensitive dyes for long-term recording of neural activity in hippocampus)

RN 79953-79-0 HCAPLUS

CN 1(4H)-Quinolinebutanesulfonic acid, 4-[4-(3-butyl-4-oxo-2-thioxo-5-thiazolidinyldene)-2-butenyldene]-, sodium salt (9CI) (CA INDEX NAME)



L76 ANSWER 6 OF 8 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1998:214017 HCAPLUS

DN 129:3193

ED Entered STN: 16 Apr 1998

TI Functional organization of rat olfactory bulb glomeruli revealed by optical imaging

AU Keller, Asaf; Yagodin, Sergey; Aroniadou-Anderjaska, Vassiliki; Zimmer, Lee A.; Ennis, Mathew; Sheppard, Norman F., Jr.; Shipley, Michael T.

CS Department of Anatomy and Neurobiology and the Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD, 21201, USA

SO Journal of Neuroscience (1998), 18(7), 2602-2612

CODEN: JNRSDS; ISSN: 0270-6474

PB Society for Neuroscience

DT Journal

LA English

CC 13-6 (Mammalian Biochemistry)

AB The functional organization and synaptic physiol. of olfactory bulb glomeruli were studied in rat in vitro slice preps. stained with the voltage-sensitive dye RH-155. Optical signals were recorded with a 100-element photodiode array at high temporal resolution. Pharmacol. and ionic manipulations were used to investigate synaptic responses to stimulation of the olfactory nerve layer (ONL). ONL stimulation evoked a sodium-mediated compound action potential that propagated across the ONL and invaded individual glomeruli. This presynaptic volley evoked calcium-dependent synaptic responses the amplitudes of which were largest within the glomerular layer (GL); smaller amplitude responses were recorded in deeper layers of the olfactory bulb. Synaptic responses in the GL were attenuated by the non-NMDA ionotropic glutamate receptor antagonist CNQX; the residual component was suppressed by the NMDA glutamate receptor antagonist AP-5. The GABAA receptor antagonist bicuculline methiodide had little effect, whereas the GABAB receptor agonist baclofen dramatically attenuated ONL-evoked synaptic responses. The effects of baclofen were reversed by the GABAB receptor antagonist CGP35348. Paired-pulse depression of ONL-evoked synaptic responses in the GL was partially reversed by CGP35348. These findings

suggest that olfactory **nerve** axons release glutamate to activate both NMDA and non-NMDA receptors on GL neurons, that GABAA receptor-mediated inhibition has little effect on these responses, and that GABAB receptor-mediated inhibition may act presynaptically on olfactory **nerve** axons to modulate their inputs to olfactory bulb neurons.

- ST glutamate NMDA receptor olfactory bulb glomerulus; GABA receptor synaptic neurotransmission olfactory bulb; sodium calcium synaptic neurotransmission olfactory bulb
- IT GABA receptors
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(GABAB; synaptic responses to stimulation of olfactory nerve layer in relation to)
- IT Glutamate receptors
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(NMDA-binding; synaptic responses to stimulation of olfactory nerve layer in relation to)
- IT Brain
(olfactory bulb, glomerulus; functional organization of rat olfactory bulb glomeruli, synaptic responses to stimulation of olfactory nerve layer)
- IT Nerve
(olfactory; functional organization of rat olfactory bulb glomeruli, synaptic responses to stimulation of olfactory nerve layer)
- IT Glutamate receptors
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(synaptic responses to stimulation of olfactory nerve layer in relation to)
- IT Neurotransmission
(synaptic; functional organization of rat olfactory bulb glomeruli, synaptic responses to stimulation of olfactory nerve layer)
- IT 7440-23-5, Sodium, biological studies 7440-70-2, Calcium, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(synaptic responses to stimulation of olfactory nerve layer in relation to)

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L76 ANSWER 7 OF 8 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1997:283109 HCAPLUS

ED Entered STN: 03 May 1997

TI Optical recording of neural signals evoked by greater superficial petrosal nerve stimulation in rat

AU Yanaura, Mamiko; Yamada, Satoshi; Shiono, Satoru; Nakashima, Michio

CS ADVANCED TECHNOLOGY R and D CENTER, MITSUBISHI ELECTRIC CORPORATION, HYOGO, 661, Japan

SO Comparative Biochemistry and Physiology, A: Physiology (1997),
117A(2), 183-190
CODEN: CBPAB5; ISSN: 0300-9629

PB Elsevier
DT Journal
LA English
AB Elec. responses to greater superficial petrosal (GSP) **nerve**
stimulation in a rat geniculate ganglion (GG) preparation were assessed by
simultaneous multi-site **optical** recording. The GG/GSP
nerve preps. were dissected out and were stained with a
voltage-sensitive dye (RH155). Application of depolarizing
square pulses to the GSP **nerve** fibers using a suction electrode
evoked **optical** (absorbance) signals that were recorded
simultaneously from many contiguous regions using a 24 x 24
photodiode matrix array with 448 active elements. Those **optical**
signals were observed along the left half area of the GSP **nerve**.
As the distance from the site of stimulation increased, the
optical signals appeared to conduct with increasing time-delay.
From the relationship between the peak latency and distance, the
conduction velocity was estimated to be about 0.4 m/s. Tetraethylammonium
affected the duration of the **optical** signals, and the signals
disappeared in solns. containing tetrodotoxin (TTX) or in Na⁺-deficient solns.
The **optical** signals evoked by the GSP **nerve**
stimulation are considered to be due to the action potentials propagating
along the GSP of unmyelinated axons.

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L76 ANSWER 8 OF 8 HCAPLUS COPYRIGHT 2005 ACS on STN
AN 1996:12103 HCAPLUS
DN 124:113606

ED Entered STN: 05 Jan 1996

TI High-speed optical imaging of afferent flow through rat olfactory bulb slices: voltage-sensitive dye signals reveal periglomerular cell activity

AU Senseman, David M.

CS Div. Life Sci., Univ. Texas, San Antonio, TX, 78249, USA

SO Journal of Neuroscience (1996), 16(1), 313-24
CODEN: JNRSDS; ISSN: 0270-6474

PB Oxford University Press

DT Journal

LA English

CC 13-6 (Mammalian Biochemistry)

AB Fast, multiple-site **optical** recording and video imaging techniques were combined to visualize the olfactory processing stream as it flowed through rat olfactory bulb slices stained with the voltage-sensitive dye **RH155**. A 464-element photodiode detector array was used to record the voltage-sensitive dye signals. Focal elec. stimulation of the olfactory **nerve** layer evoked relatively large **optical** responses in the olfactory **nerve** and glomerular layers but only small responses within the external plexiform layer. With paired-pulse stimulation, glomerular attenuation was evident in signals recorded from the glomerular and external plexiform layers but not from the olfactory **nerve** layer. At very high recording speeds (<0.2 ms/frame), the presynaptic component of the olfactory processing stream could be followed as it flowed through the olfactory **nerve** layer and into the glomerular layer, where its amplitude rapidly declined. This decline was followed by a reciprocal rise in a postsynaptic depolarization that was largely restricted to the glomerular layer. Spatiotemporal interactions between overlapping afferent streams within the glomerular layer were observed and partially characterized. The **optically** recorded glomerular layer response was largely resistant to bath application of GABAA receptor antagonists but was sensitive to manipulations of external chloride concentration and to bath application of a stilbene derivative, 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonic acid known to block Cl⁻ conductances. It is suggested that the voltage-sensitive dye signals recorded from the glomerular layer reflect activity in periglomerular cells and that Cl⁻ efflux through non-GABAA chloride channels contributes to the postsynaptic depolarization of these cells after olfactory **nerve** stimulation.

ST neurotransmission olfactory bulb chloride channel

IT Neurotransmission
(voltage-sensitive dye signals in high-speed optical imaging of afferent flow through rat olfactory bulb slices with respect to periglomerular cell activity and chloride conductance)

IT Ion channel
(chloride, voltage-sensitive dye signals in high-speed optical imaging of afferent flow through rat olfactory bulb slices with respect to periglomerular cell activity and chloride conductance)

IT Brain
(olfactory bulb, voltage-sensitive dye signals in high-speed optical imaging of afferent flow through rat olfactory bulb slices with respect to periglomerular cell activity and chloride conductance)

IT 16887-00-6, Chloride, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(voltage-sensitive dye signals in high-speed optical imaging of afferent flow through rat olfactory bulb slices with respect to periglomerular cell activity and chloride conductance)

=> d all hitstr tot 177

L77 ANSWER 1 OF 3 HCAPLUS COPYRIGHT 2005 ACS on STN
 AN 2004:267152 HCAPLUS
 DN 140:276160
 ED Entered STN: 01 Apr 2004
 TI **Polymethine** organic dye compd for inducing receptor potential in
 response to photostimulation in the **optic nerve**
 IN **Matsuo, Toshihiko; Kan-oh, Yasufumi; Suga,**
Sadaharu
 PA **Kabushiki Kaisha Hayashibara Seibutsu Kagaku Kenkyujo, Japan**
 SO U.S. Pat. Appl. Publ., 7 pp.
 CODEN: USXXCO
 DT Patent
 LA English
 IC ICM A61K049-00
 ICS C12Q001-00; C07D417-02; C07D043-02
 INCL 424009600; 435004000; 548181000; 548454000
 CC 63-5 (Pharmaceuticals)
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
PI	US 2004062713	A1	20040401	US 2003-673487	20030930	<--
	JP 2004121292	A2	20040422	JP 2002-285784	20020930	<--
PRAI	JP 2002-285784	A	20020930			<--

CLASS

	PATENT NO.	CLASS	PATENT FAMILY CLASSIFICATION CODES	
US	2004062713	ICM	A61K049-00	
		ICS	C12Q001-00; C07D417-02; C07D043-02	
		INCL	424009600; 435004000; 548181000; 548454000	
US	2004062713	NCL	424/009.600; 435/004.000; 548/181.000; 548/454.000	
		ECLA	A61K041/00; C07D209/14; C07D215/12; C07D231/22; C07D263/56B; C07D277/10; C07D311/82; C07D413/06+263+233; C07D417/06+277+231; C07D417/06+277B+215; C07D491/10+311B+209B	<--
JP	2004121292	FTERM	4C081/AB21; 4C081/BB03; 4C081/CE11	<--
AB	Disclosed is an agent for inducing receptor potential, which comprises an organic dye compound capable of inducing/evoking receptor potential in response to photostimulation in the optic nerve , wherein the organic dye compound is a polymethine organic dye compound Also disclosed is a substituent material for the retina comprising the agent.			
ST	polymethine org dye compd receptor potential photostimulation optic nerve			
IT	Polyenes RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses) (conjugated; polymethine organic dye compd for inducing receptor potential in response to photostimulation in optic nerve)			
IT	Drug delivery systems (ophthalmic; polymethine organic dye compd for inducing receptor potential in response to photostimulation in optic nerve)			
IT	Nerve (optic ; polymethine organic dye compd for inducing receptor potential in response to photostimulation in optic nerve)			
IT	Receptors RL: BSU (Biological study, unclassified); BIOL (Biological study) (potential; polymethine organic dye compd for inducing receptor			

potential in response to photostimulation in optic nerve)

L77 ANSWER 2 OF 3 HCAPLUS COPYRIGHT 2005 ACS on STN
 AN 2001:467447 HCAPLUS
 DN 135:327644
 ED Entered STN: 28 Jun 2001
 TI Effect of neonatal capsaicin treatment on neural activity in the medullary dorsal horn of neonatal rats evoked by electrical stimulation to the trigeminal afferents: an optical, electrophysiological, and quantitative study
 AU Takuma, S.
 CS Department of Dental Anesthesiology, Hokkaido University Graduate School of Dental Medicine, Sapporo, 060-8586, Japan
 SO Brain Research (2001), 906(1,2), 1-12
 CODEN: BRREAP; ISSN: 0006-8993
 PB Elsevier Science B.V.
 DT Journal
 LA English
 CC 2-8 (Mammalian Hormones)
 AB To elucidate which glutamate receptors, NMDA or non-NMDA, have the main role in synaptic transmission via unmyelinated afferents in the trigeminal subnucleus caudalis (the medullary dorsal horn), and to examine the early functional effects of neonatal capsaicin treatment to the subnucleus caudalis, **optical** recording, field potential recording, and quant. study using electron micrographs were employed. A medulla oblongata isolated from a rat 5-7 days old was sectioned horizontally 400- μ m thick or parasagittally and stained with a voltage-sensitive dye, **RH482** or **RH795**. Single-pulse stimulation with high intensity to the trigeminal afferents evoked **optical** responses mainly in the subnucleus caudalis. The **optical** signals were composed of two phases, a fast component followed by a long-lasting component. The spatiotemporal properties of the **optical** signals were well correlated to those of the field potentials recorded simultaneously. The fast component was eliminated by 6-cyano-7-nitroquinoxaline-2,3-dione (**CNQX**; 10 μ M), while the long-lasting component was not. The latter increased in amplitude under a condition of low Mg^{2+} but was significantly reduced by DL-2-amino-5-phosphonovaleric acid (**AP5**; 30 μ M). Neonatal capsaicin treatment also reduced the long-lasting component markedly. In addition, the decreases in the ratio of unmyelinated axons to myelinated axons and in the ratio of unmyelinated axons to Schwann cell subunits of trigeminal **nerve** roots both showed significant differences ($P < 0.05$, Student's t-test) between the control group and the neonatal capsaicin treatment group. This line of evidence indirectly suggests that synaptic transmission via unmyelinated afferents in the subnucleus caudalis is mediated substantially by NMDA glutamate receptors and documented that neonatal capsaicin treatment induced a functional alteration of the neural transmission in the subnucleus caudalis as well as a morphol. alteration of primary afferents within several days after the treatment.
 ST neonate capsaicin medullary dorsal horn neural activity; synaptic neurotransmission glutamate receptor trigeminal subnucleus caudalis neonate capsaicin
 IT Glutamate receptors
 RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
 (NMDA-binding; effect of neonatal capsaicin treatment on neural activity in the medullary dorsal horn of neonatal rats evoked by elec. stimulation to the trigeminal afferents)
 IT Neurotransmission

(synaptic; effect of neonatal capsaicin treatment on neural activity in the medullary dorsal horn of neonatal rats evoked by elec. stimulation to the trigeminal afferents)

IT Brain

(trigeminal nucleus, caudal; effect of neonatal capsaicin treatment on neural activity in the medullary dorsal horn of neonatal rats evoked by elec. stimulation to the trigeminal afferents)

IT Nerve

(trigeminal; effect of neonatal capsaicin treatment on neural activity in the medullary dorsal horn of neonatal rats evoked by elec. stimulation to the trigeminal afferents)

IT 404-86-4, Capsaicin 7439-95-4, Magnesium, biological studies

RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)

(effect of neonatal capsaicin treatment on neural activity in the medullary dorsal horn of neonatal rats evoked by elec. stimulation to the trigeminal afferents)

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L77 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2005 ACS on STN

AN 1999:296512 HCAPLUS

ED Entered STN: 14 May 1999

TI Altered spatial patterns of functional thalamocortical connections in the barrel cortex after neonatal infraorbital **nerve** cut revealed by **optical** recording

AU Higashi, S.; Crair, M. C.; Kurotani, T.; Inokawa, H.; Toyama, K.

CS Department of Physiology, Kyoto Prefectural University of Medicine, Kyoto, 602-8566, Japan

SO Neuroscience (Oxford) (1999), 91(2), 439-452

CODEN: NRSCDN; ISSN: 0306-4522

PB Elsevier Science Ltd.

DT Journal

LA English

AB In rodents, the somatosensory cortex has a cell aggregation cluster termed the barrel, reflecting a whisker vibrissa, and this barrel formation is disrupted by infraorbital **nerve** cut at birth. In the present study, we prepared thalamocortical slice prepns. from rats that received infraorbital **nerve** cut either at birth or at postnatal day (P) 7 and those from normal rats, recorded the **optical** response reflecting neural excitation in the somatosensory cortex with a voltage-sensitive dye (RH482) and compared the **optical** responses from lesioned rats with those from normal rats. In normal rats at P10, the **optical** response elicited elec. by thalamic stimulation propagated to the cortex, and then several patchy clusters appeared in layer IV. The size and location of these patchy responses precisely matched either barrels identified by cytochrome oxidase staining or terminal arbors of thalamocortical axons stained with biotinylated dextran amine. In contrast, at P10 in P0-lesioned rats, clusters having a wider horizontal width but smaller amplitude than those seen in normal rats appeared in layer IV. Correspondingly, neither cytochrome oxidase staining nor biotinylated dextran amine labeling of thalamocortical axons showed any barrel-like clusters or glomerular axon terminals. Likewise, at P5-P6, the tangential width of clusters in layer IV were larger than that in normal rats. At P10 in P7-lesioned rats, small cluster-matched barrels were seen in the **optical** response as well as in normal rats. These results suggest that P0 infraorbital **nerve** cut interrupted segregation of functional synapses into the barrels and retarded the maturation of thalamocortical transmission.

RE.CNT 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD

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L80 ANSWER 1 OF 1 USPATFULL on STN

AN 92:59668 USPATFULL

TI Optical sensor

IN Koshiishi, Kiyozou, Sagamihara, Japan

Shinohara, Etsuo, Hachioji, Japan

Shimomura, Masatsugu, Koganei, Japan

PA Olympus Optical Co., Ltd., Tokyo, Japan (non-U.S. corporation)

PI US 5132095 19920721

AI US 1990-589492 19900927 (7)

PRAI JP 1989-259611 19891004

DT Utility

FS Granted

EXNAM Primary Examiner: Warden, Robert J.; Assistant Examiner: Trembley, T. A.

LREP Frishauf, Holtz, Goodman & Woodward
CLMN Number of Claims: 24
ECL Exemplary Claim: 1
DRWN 4 Drawing Figure(s); 2 Drawing Page(s)
LN.CNT 468

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB An optical sensor for detecting a specific substance in a solution, based on optical changes, includes a substrate and a thin membrane formed on the substrate. The membrane is formed of an ion complex material of an ionic amphipathic compound with a polymer having ionic groups of the opposite electrical charge, a potential-sensitive dye and a substance-selective compound.

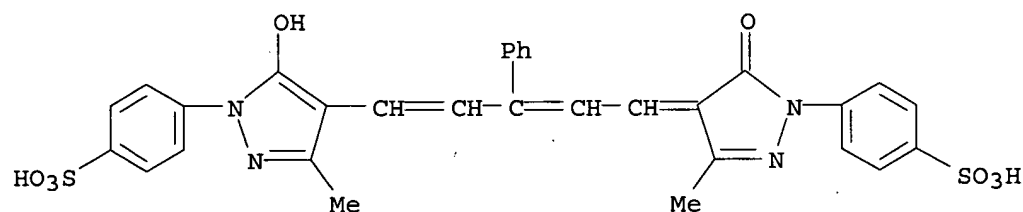
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

IT 135806-37-0

(optical sensor containing, thin-membrane, for analyzing solns.)

RN 135806-37-0 USPATFULL

CN Benzenesulfonic acid, 4-[4,5-dihydro-4-[5-[5-hydroxy-3-methyl-1-(4-sulfophenyl)-1H-pyrazol-4-yl]-3-phenyl-2,4-pentadienylidene]-3-methyl-5-oxo-1H-pyrazol-1-yl]-, trisodium salt (9CI) (CA INDEX NAME)



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Evaluation of Voltage-Sensitive Dyes for Long-Term Recording of Neural Activity in the Hippocampus

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Abstract. We searched for an optimal voltage-sensitive dye for optical measurements of neural activity in the hippocampal slice by evaluating several merocyanine-rhodanine and oxonol dyes. The wavelength dependence (action spectra), pharmacological effects of staining, signal size, signal-to-noise ratio, and the utility of the dyes for long-term continuous recording were examined for four merocyanine-rhodanine dyes (NK2761, NK2776, NK3224 and NK3225), which had been reported to be optimal in embryonic nervous systems, and for two oxonol dyes (NK3630 (RH482) and NK3041 (RH155)), which have been among the most popular potentiometric probes for the hippocampal slice preparation. NK2761, NK3224 and NK3225 provided large signal-to-noise ratios, and proved to be useful for optical recordings lasting several hours. NK3630 was most suitable for long-term recording, although the signal-to-noise ratio was slightly inferior to that of the merocyanine-rhodanines. Using NK3630 (RH482) on the hippocampal slice preparation, we demonstrate here that long-term potentiation can be monitored stably for more than 8 hr.

Key words: Optical recording — Voltage-sensitive dye — Dye screening — Merocyanine-rhodanine — Hippocampal slice — Long-term potentiation

Introduction

Hippocampal slices constitute an organized laminar structure suitable for a physiological analysis of synaptic connections among neurons. The hippocampus is also used as an excellent model system for the analysis of long-term potentiation (LTP), which is considered to be a fundamental cellular mechanism responsible for the

phenomena of learning and memory (Tsumoto, 1992; Bliss & Collingridge, 1993). For these investigations, conventional electrophysiological measurements have usually been applied, although they have some technical limitations: only a restricted number of electrodes can be placed in the preparation, and intracellular recording can be made only from large elements (e.g., cell bodies and large dendrites) and for relatively short durations.

Optical recording techniques with voltage-sensitive dyes have provided a powerful means for monitoring neural electrical activity offering two principal advantages over conventional electrophysiological techniques. One is that it is possible to monitor intracellular membrane potential changes directly and noninvasively. The other is that multiple sites of a preparation can be monitored simultaneously (for reviews see Cohen & Salzberg, 1978; Salzberg, 1983; Grinvald et al., 1988; Kamino, 1990, 1991). Since the first optical recording study in the hippocampal slice (Grinvald, Mankner & Segal, 1982), many investigations have been devoted to this preparation, using absorption (Barish et al., 1996; Iijima et al., 1996; Nakagami, Saito & Matsuki, 1997; Sekino et al., 1997; Kojima et al., 1999), and fluorescent (Saggau, Galvan & Bruggencate, 1986; Albowitz & Kuhnt, 1991; Iijima et al., 1996) voltage-sensitive dyes (for a review see Ebner & Chen, 1995). Most of the recent works used oxonol dyes, such as RH155 and RH482, and they demonstrated that these absorption dyes provide usable signals (Barish et al., 1996; Iijima et al., 1996; Nakagami et al., 1997; Sekino et al., 1997; Kojima et al., 1999). On the other hand, we have previously reported that, in the embryonic nervous system, merocyanine-rhodanine dyes are better than the oxonols (Momose-Sato et al., 1995).

The choice of optimal dyes is an important consideration in optical recordings. Since transmission measurements are usually more advantageous than fluorescence in brain slice preparations (Grinvald et al., 1988; Wu & Cohen, 1993), we have compared the properties of

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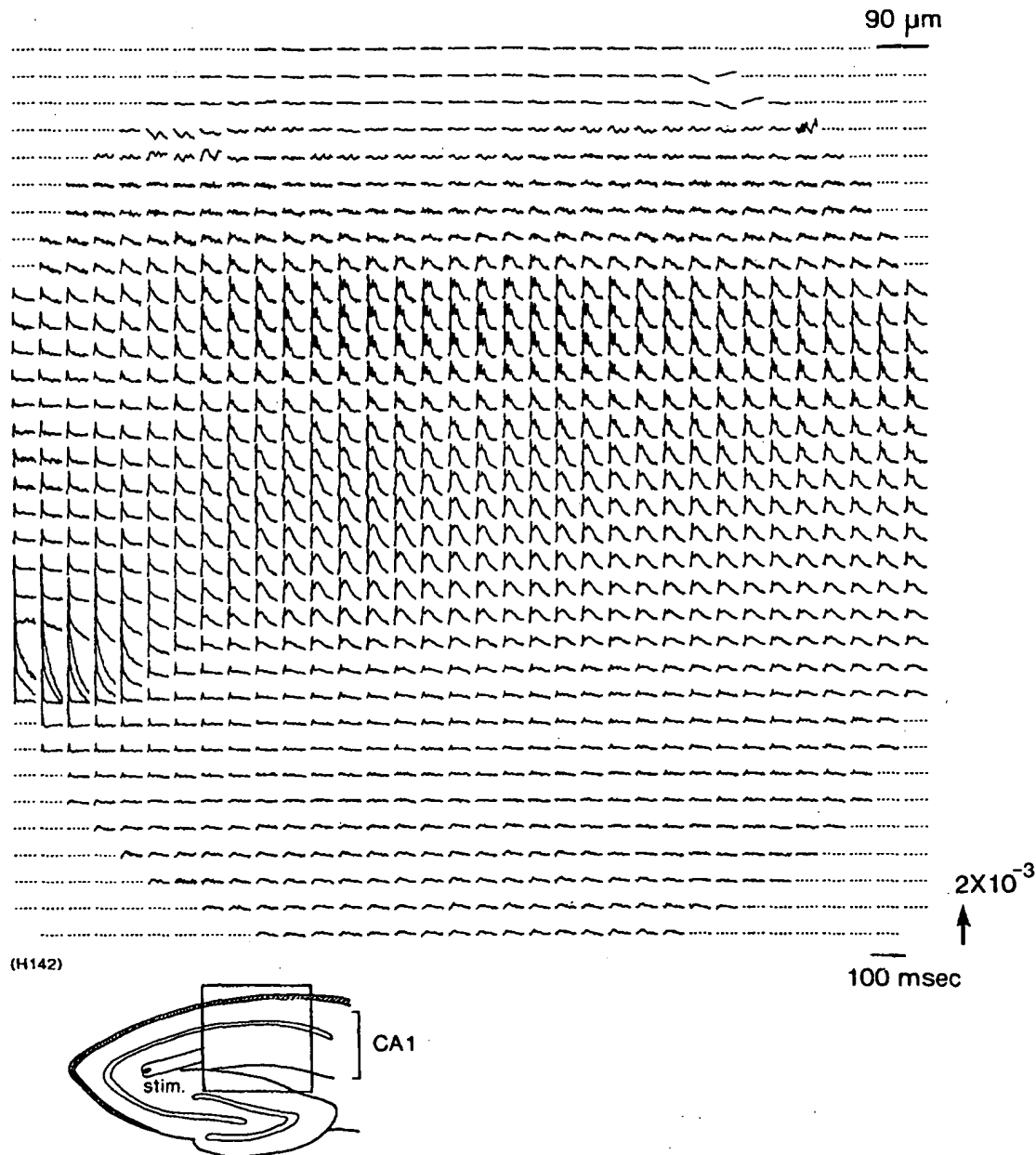


Fig. 1. Multiple-site optical recording of neural responses in a hippocampal slice preparation stained with an oxonol dye NK3630 (RH482) (0.5 mg/ml). The optical signals were evoked by applying a square current pulse (150 μ A/250 μ sec) to the Schaffer collateral pathway with a bipolar electrode. The evoked optical signals were detected using a 34 \times 34 matrix photodiode array from the region indicated by a square in the lower inset. Four trials were averaged. The direction of the arrow on the right of the recording indicates a decrease in transmitted light (increase in dye absorption), and the length of the arrow represents the stated value of the fractional change (the change in the light intensity divided by DC-background-intensity).

two major classes of absorption dyes, e.g., the merocyanine-rhodanines and the oxonols, in the present experiment. The second aim of this study is to evaluate the utility of the various voltage-sensitive dyes for long-term monitoring of hippocampal neural activity. Recently, a late phase of LTP, which lasts longer than 4 hr, has been

distinguished from an early phase of LTP, and the importance of *in vitro* investigations with long-term recording has been emphasized (Abraham & Otani, 1991; Frey et al., 1988, 1996). Optical recording of intracellular membrane potential changes from multiple regions would be a useful tool for the study of LTP.

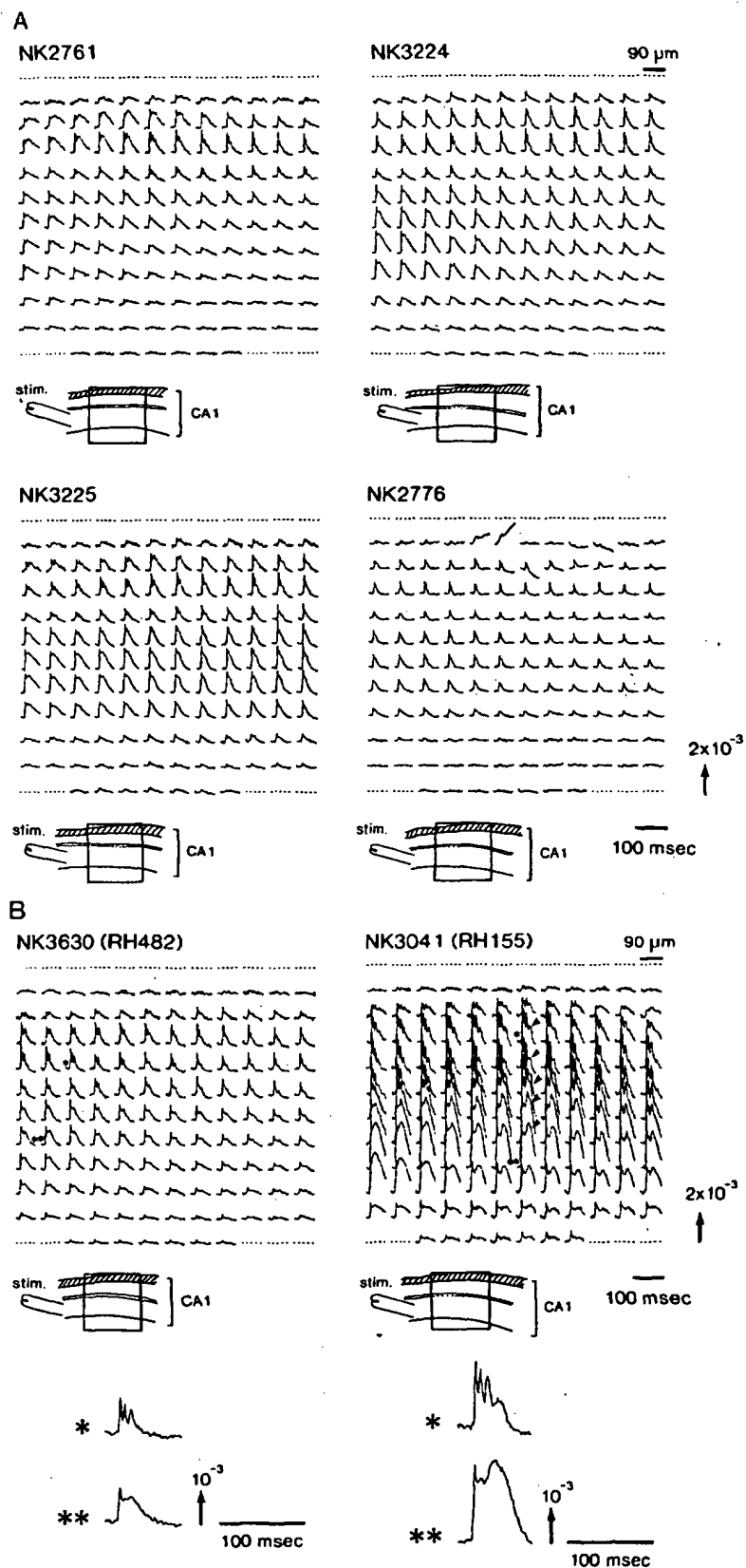


Fig. 2. Multiple-site optical recordings of neural responses in hippocampal slice preparations stained with four merocyanine-rhodanine dyes (A) NK2761, NK3224, NK3225 and NK2776, and two oxonol dyes (B) NK3630 (RH482) and NK3041 (RH155). In B, enlargements of the optical signals labeled with asterisks are presented on the bottom. The evoked optical signals were detected using a 12 × 12 matrix photodiode array from the CA1 region. In this and the following figures, two trials were averaged for the merocyanine-rhodanine dyes, and three trials were averaged for the oxonol dyes, except where noted.

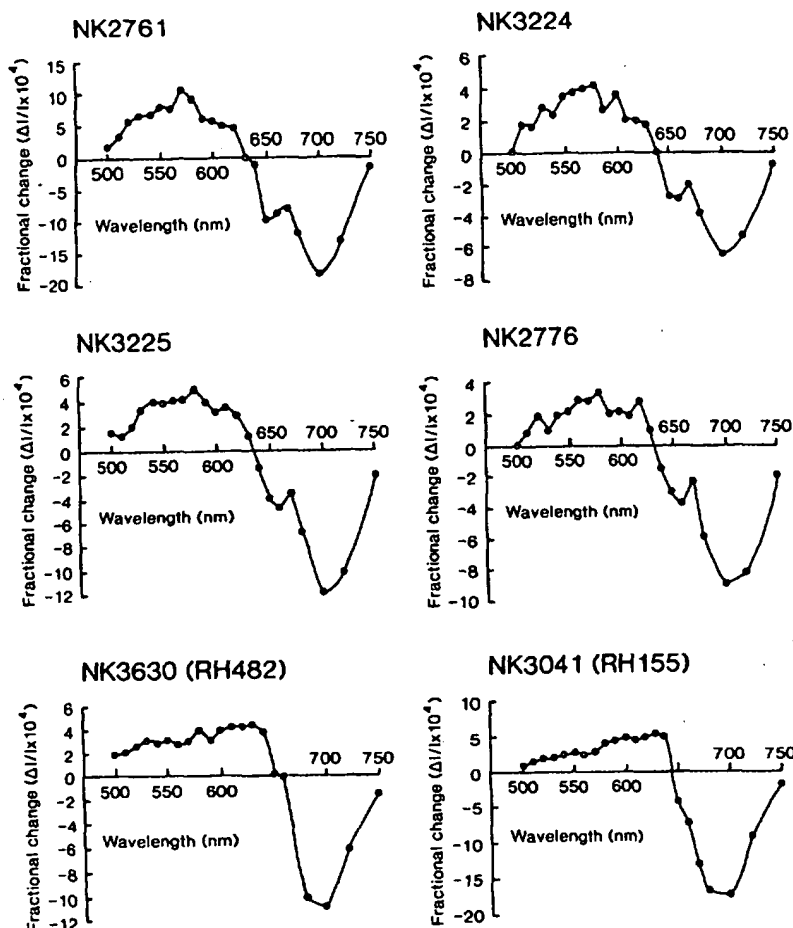


Fig. 3. Wavelength dependence of the optical signals. The amplitudes of the largest fast optical signal were plotted against the wavelength of the incident light.

Materials and Methods

HIPPOCAMPAL SLICE PREPARATIONS

Male Wistar rats (Saitama experimental animals supply, Saitama, Japan) 8–10 weeks of age were decapitated under ether anesthesia. Brains were quickly removed and cooled in iced artificial cerebrospinal fluid (ACSF). The solution contained (in mM): NaCl 124, KCl 5, MgSO₄ 1, CaCl₂ 2.5, NaH₂PO₄ 1.25, NaHCO₃ 22 and glucose 10, and was continuously bubbled with a mixture of 95% O₂ and 5% CO₂ (pH 7.4). Transverse slices of hippocampus, 300 μ m thick, were prepared using a rotorslicer (DTY-8700, Dosaka EM, Kyoto, Japan). The slices were maintained at room temperature (26–30°C) for at least 1 hr before use. The slice was transferred to a recording chamber and was continuously perfused with ACSF at a rate of 1–5 ml/min (usually 1 ml/min) at 30–32°C.

ELECTRICAL STIMULATION

The Schaffer collateral pathway was stimulated using a bipolar tungsten electrode. A square current pulse (100–400 μ A/250 μ sec), which evoked nearly maximum responses in the CA1 region, was delivered at 0.05 Hz. In LTP experiments, the current intensity of test pulses was adjusted so as to elicit an excitatory postsynaptic potential (EPSP)-related slow optical signal of 30–50% of its maximal amplitude. LTP

lasting longer than 5 hr was induced by tetanic stimulation using either three stimulus trains of 100 pulses (100 Hz/1 sec duration) with 10 min intertrain intervals (Frey et al., 1988, 1996), or 50 trains of 10 pulses (400 Hz/25 msec duration) presented as 10 bursts of 5 trains at 1 Hz (1 min between bursts) (Otani et al., 1989; Abraham et al., 1993).

DYE STAINING

The slice was stained for 5 min in ACSF solution to which 0.2–0.5 mg/ml of the dye (usually 0.5 mg/ml) was freshly dissolved. After the staining, the preparation was washed with perfusion of normal ACSF, and was kept in the dark. The dyes used in the present experiment were as follows. Merocyanine-rhodanine: NK2761, NK2776, NK3224, NK3225. These dyes have been reported to be optimal for monitoring neural activity from early embryonic nervous systems (Momose-Sato et al., 1995). Oxonol: NK3041 (RH155), NK3630 (RH482). These dyes have been most frequently used in recent optical studies in hippocampal and other slice preparations (Konnerth, Obaid & Salzberg, 1987; Barish et al., 1996; Iijima et al., 1996; Nakagami et al., 1997; Sekino et al., 1997). The chemical structures of these dyes have been described previously (Konnerth et al., 1987; Momose-Sato et al., 1995); the dyes were purchased from Kankoh-Shikiso Kenkyusho (Okayama, Japan).

OPTICAL RECORDING

The preparation chamber was mounted on the stage of an Olympus Vanox microscope (Type AHB-L-1). Bright-field illumination was

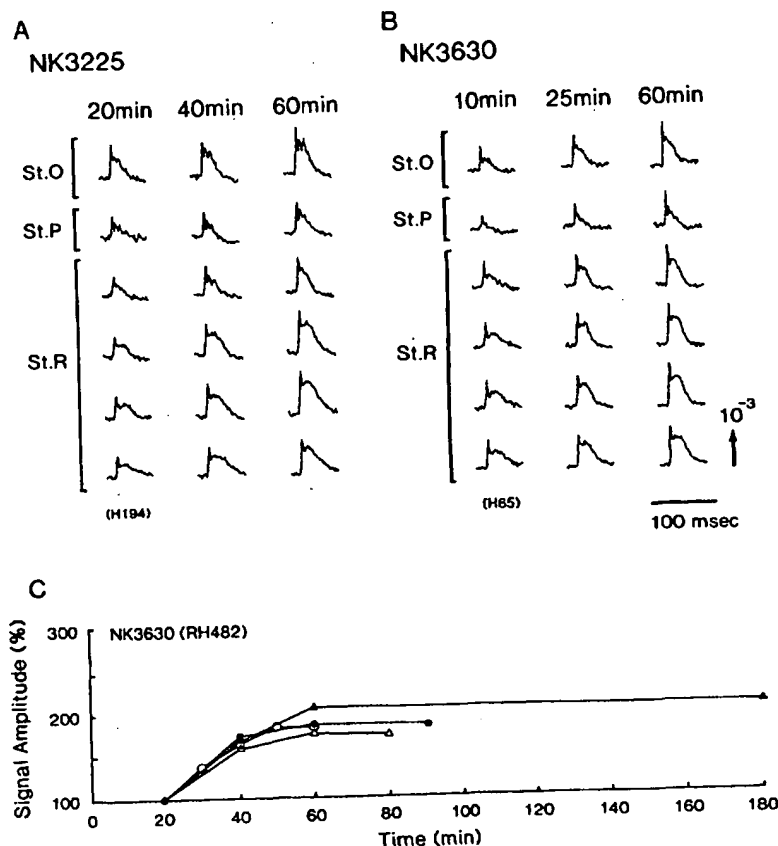


Fig. 4. Time-dependent change in the optical signals after staining. Enlargements of the optical signals obtained from the stratum oriens (St. O), stratum pyramidale (St. P) and stratum radiatum (St. R) are presented. Recordings were made 20, 40 and 60 min after the staining with NK3225 (A), and 10, 25 and 60 min after the staining with NK3630 (B). The dye concentration was 0.5 mg/ml. In C, normalized amplitudes of the slow optical signals (mean of eight signals) detected from the stratum radiatum are plotted against the time. Different symbols correspond to different preparations ($n = 4$).

provided by a 300-W tungsten-halogen lamp (Type JC-24V-300W, Kondo-Philips, Tokyo, Japan) driven by a stable dc-power supply. Incident light was made quasimonochromatic by an interference filter (703 ± 15 nm; Asahi Spectra, Tokyo, Japan) placed between the light source and the preparation. A microscope objective ($\times 10$, S plan Apo, 0.4 n.a.) and a photographic eyepiece ($\times 1.67$, $\times 2.5$ or $\times 3.3$) formed a magnified ($\times 16.7$, $\times 25$ or $\times 33$) real image of the preparation at the image plane. The transmitted light intensity at the image plane was detected using a multi-element silicon photodiode matrix array. In the present experiments, we used two optical recording systems, which were constructed in this laboratory. One is a 1020-site optical recording system with a 34×34 -element silicon photodiode array (Hamamatsu Photonics, Hamamatsu, Japan) (for details see Hirota et al., 1995; Sato et al., 1998). The outputs from 1020 elements were fed into amplifiers via current-to-voltage converters and then passed to 32 sets of 32-channel analog multiplexers. Each output from the multiplexers was fed into a subranging type analog-to-digital (AD) converter system with a resolution of 18 bits and was sent to a computer. Another recording system is a 128-channel multiple-site optical recording system using a 12×12 -element silicon photodiode array (MD-144-4PV, Centronic, Croydon, UK) (for details see Kamino, 1990, 1991; Momose-Sato et al., 1998). The output of each detector in the diode array was passed to an amplifier (AC coupling = 3 sec) via a current-to-voltage converter. The amplified outputs from 127 elements of the detector were recorded simultaneously on the videotape of a 128-channel data recording system (RP-890 series, NF Electronic Instruments, Yokohama, Japan) and were passed to a computer. The time resolution of these systems was ≈ 1 msec. In each recording, 2–4 trials were averaged, and no offline filtering was used.

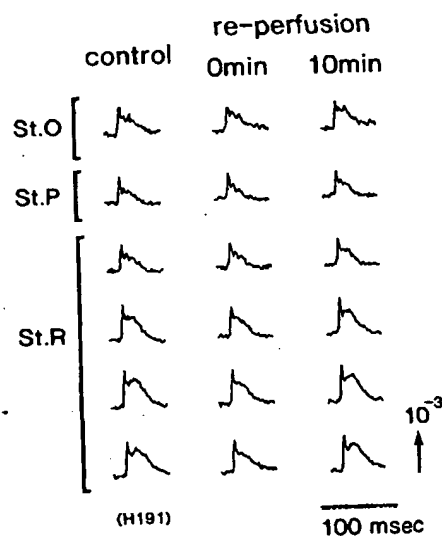


Fig. 5. Effects of stopping perfusion on the optical signals. Recordings were made before perfusion stop for 5 min (control), just after reperfusion (0 min) and 10 min after reperfusion (10 min). The preparation was stained with NK3630, and the control recording was made 3 hr after the staining.

Table 1. Rate of increase in signal size after staining

Dye	Preparation reference	Dye concentration (mg/ml)	Perfusion rate (ml/min)	Pre-incubation (hr)	90% recovery time (min)	
					Fast signal	Slow signal
NK2761	H181	0.5	1	3	61	60
	H182	0.2	1	5	59	60
	H184	0.2	5	4	52	59
	H192	0.2	5	4	36	50
	H190	0.2	5	7.5	56	50
NK2776	H195	0.5	1	1	97	93
	H197	0.5	1	1	112	120
NK3224	H196	0.5	1	1	86	96
	H201	0.5	1	1	77	60
NK3225	H194	0.5	1	1	104	108
	H199	0.5	1	1	80	94
NK3630 (RH482)	H107	0.5	1	1	42	48
	H132	0.5	1	2	30	41
	H113	0.5	1	6	40	38
	H65	0.5	6	1	35	42
	H131	0.5	6	1	45	42
NK3041 (RH155)	H198	0.5	1	1	<10	<10
	H193	0.5	1	1	<20	<20
	H141	0.5	1	5	<20	<20

The time required for attaining 90% of the maximum signal amplitude was evaluated for the fast and slow signals detected from the stratum radiatum. The values were measured from the plots shown in Fig. 4C.

Results

OPTICAL RESPONSES EVOKED BY SCHAFER COLLATERAL STIMULATION

Figure 1 shows an example of optical recordings made in a hippocampal slice preparation stained with an oxonol dye, NK3630 (RH482). The signals were evoked by stimulation of the Schaffer collateral pathway, and the recording was made by averaging four trials using a 1020-element photodiode array. The magnification was $\times 33$, and each pixel (element) of the array detected light transmitted by a square region ($45 \times 45 \mu\text{m}^2$) of the preparation.

In this recording, the optical signals were evoked in a wide area of the CA1 region. The signals detected at the stratum radiatum consisted of two components, viz., fast spike-like and slow signals. It has been reported that, using the voltage-sensitive dyes (e.g., RH482), the fast and slow components represent action potentials and excitatory postsynaptic potentials (EPSPs), respectively, because the slow signal is eliminated in Ca^{2+} -free solution and the fast signal is blocked by tetrodotoxin (Grinvald et al., 1982; Nakagami et al., 1997). Similar results were obtained in the present experiment. The slow signal was also reduced by APV (DL-2-amino-5-phosphonovaleric acid; $190 \mu\text{M}$) and CNQX (6-cyano-7-nitro-

quinoxaline-2, 3-dione; $5 \mu\text{M}$). At the strata pyramidale and oriens, multiple spike-like optical signals were triggered, which have been described as reflecting the action potential discharge (Grinvald et al., 1982). Because the optical signals near the stimulation electrode were contaminated with electrotonic potential-related signals, in the following experiments, we focused on the region which is $400\text{--}4500 \mu\text{m}$ from the tip of the electrode.

Figure 2 shows typical examples of original recordings made in a CA1 region stained with four merocyanine-rhodanine dyes (NK2761, NK3224, NK3225 and NK2776) (Fig. 2A), and two oxonol dyes (NK3630 (RH482) and NK3041 (RH155)) (Fig. 2B). For these recordings, a 12×12 -element photodiode array with a magnification of $\times 16.7$ was used. In this and the subsequent figures, two trials were averaged for the merocyanine-rhodanine dyes, and three trials were averaged for the oxonol dyes, except where noted. In the recordings shown in Fig. 2, the waveforms of the optical signals were almost identical for the various dyes, with the exception of NK3041. The signals provided by NK3041 appear to have another slow component with long duration (arrowheads in Fig. 2B). Konnerth et al. (1987) reported that RH155 exhibited a large slow wave in skate cerebellar slices, which is the result of an exceptionally high affinity of this dye for glial cell membrane, which monitors $[\text{K}^+]_o$. The large slow component observed in

Table 2. Signal size and signal-to-noise ratio

Dye	Preparation reference	Stratum radiatum						Stratum pyramidale			Stratum oriens		
		Fast signal			Slow signal			Fast signal			Fast signal		
		$\Delta I/I$ Max ($\times 10^{-4}$)	I (arbitrary unit)	S/N	$\Delta I/I$ Max ($\times 10^{-4}$)	I (arbitrary unit)	S/N	$\Delta I/I$ Max ($\times 10^{-4}$)	I (arbitrary unit)	S/N	$\Delta I/I$ Max ($\times 10^{-4}$)	I (arbitrary unit)	S/N
NK2761 (0.5 mg/ml)	H153	14.0	0.86	13.5	16.0	0.84	11.5	8.0	0.75	7.7	10.9	0.67	7.9
	H167	15.6	1.27	18.4	11.0	1.27	13.0	10.9	1.35	11.0	14.9	1.05	16.2
	H174	12.5	1.19	12.6	10.3	1.19	10.4	7.8	1.14	7.8	13.0	0.90	10.8
	H181	16.2	1.28	19.1	10.4	1.28	12.3	13.1	1.21	14.3	16.0	0.85	11.3
	H187	9.9	0.90	7.7	8.5	0.90	6.7	7.4	0.85	7.5	8.6	0.78	6.8
NK2761 (0.2 mg/ml)	H184	7.9	1.49	7.0	4.7	1.49	4.2	4.9	1.42	4.3	6.4	1.38	4.8
	H189	3.9	1.42	3.0	1.5	1.42	1.2	2.6	1.43	3.1	3.0	1.11	2.4
	H190	3.9	1.28	2.8	2.7	1.28	1.9	3.0	1.15	1.8	4.0	0.94	2.3
	H192	4.0	1.52	3.5	3.1	1.52	2.7	3.5	1.36	3.5	2.6	1.28	2.3
NK2776 (0.5 mg/ml)	H195	8.5	1.21	10.0	5.4	1.21	6.4	4.7	1.07	6.0	5.7	1.10	5.8
	H197	8.0	1.52	6.3	4.6	1.52	3.6	4.4	1.50	3.5	10.8	1.24	9.5
NK3224 (0.5 mg/ml)	H196	11.0	1.23	15.6	7.7	1.23	10.9	7.6	1.25	7.6	15.1	1.19	14.2
	H201	15.6	1.04	12.3	13.0	1.04	10.2	7.0	1.11	8.2	12.1	0.84	14.3
NK3225 (0.5 mg/ml)	H194	15.7	1.00	13.9	14.7	1.06	11.5	10.5	1.00	7.1	15.5	0.94	12.2
	H199	16.0	1.34	16.2	13.9	1.34	14.0	10.0	1.31	9.4	16.4	1.08	12.9
NK3630 (0.5 mg/ml) (RH482)	H69	10.1	0.78	6.5	8.8	0.78	5.7	7.5	0.73	4.2	9.5	0.58	6.7
	H105	10.9	0.60	9.0	8.2	0.60	6.8	7.4	0.70	6.1	9.5	0.49	5.5
	H110	12.1	0.66	6.4	7.0	0.66	3.7	9.5	0.76	5.5	14.0	0.50	5.8
	H113	11.8	0.71	7.2	8.7	0.71	5.3	5.0	0.72	3.0	8.7	0.49	4.2
	H132	9.5	0.51	5.5	10.0	0.51	5.8	5.5	0.51	2.9	6.0	0.35	3.5
	H142	10.5	0.59	8.1	7.8	0.59	6.0	10.6	0.59	5.6	14.0	0.45	6.5
	H175	11.0	0.76	8.6	7.0	0.76	5.5	12.5	0.76	9.3	15.4	0.47	7.3
	H188	11.0	0.62	6.5	8.0	0.62	4.7	10.1	0.58	7.1	13.9	0.42	6.1
	H191	9.0	0.86	5.3	6.5	0.86	3.8	6.4	0.71	3.6	6.0	0.56	4.0
NK3041 (0.5 mg/ml) (RH155)	H141	31.8	0.35	15.9	35.0	0.39	17.6	22.5	0.43	12.4	22.5	0.36	11.3
	H193	18.4	0.74	14.5	18.0	0.81	12.7	13.0	0.74	9.2	11.0	0.62	8.6
	H198	15.5	0.86	11.0	15.0	0.86	10.6	15.6	0.77	10.5	23.8	0.59	15.3

The signal size ($\Delta I/I$: fractional change in transmitted light intensity), the background light intensity (I) and the signal-to-noise ratio (S/N) were evaluated for the best signals obtained from the stratum radiatum, stratum pyramidale and stratum oriens. The signal-to-noise ratio was measured in a single sweep recording.

the hippocampal slice preparation might also be related to such a glial depolarization. Among the merocyanine-rhodanine dyes, NK2776 usually exhibited relatively small optical signals and fewer multiple spike discharges. This dye often aggregated after the staining, and it is possible that the staining conditions were not as good as with the other merocyanine-rhodanine dyes.

ACTION SPECTRA

Voltage-sensitive dye absorption changes are well known to be dependent on the wavelength of the incident light (Waggoner & Grinvald, 1977; Cohen & Salzberg, 1978; Kamino, Hirota & Komuro, 1989). It is also known that the action spectrum of the poten-

tial-related optical signal differs from species to species (Ross & Reichardt, 1979; Senseman & Salzberg, 1980). Figure 3 shows the action spectra of the six dyes measured in the hippocampal slice preparation. The four merocyanine-rhodanine dyes exhibited the same action spectra: the transmitted light intensity changed in the positive direction in the range of 500–630 nm, and in the negative direction in the range of 640–750 nm, with the crossover occurring at 630–640 nm. The maximum absorption changes were obtained at 700 nm and 580 nm. These characteristics were the same as those observed in embryonic nervous systems (Momose-Sato et al., 1995), but slightly different from those obtained in adult and embryonic hearts (Hirota et al., 1985; Komuro et al., 1986). On the other hand, the shape of the action spectra of the oxonol dyes

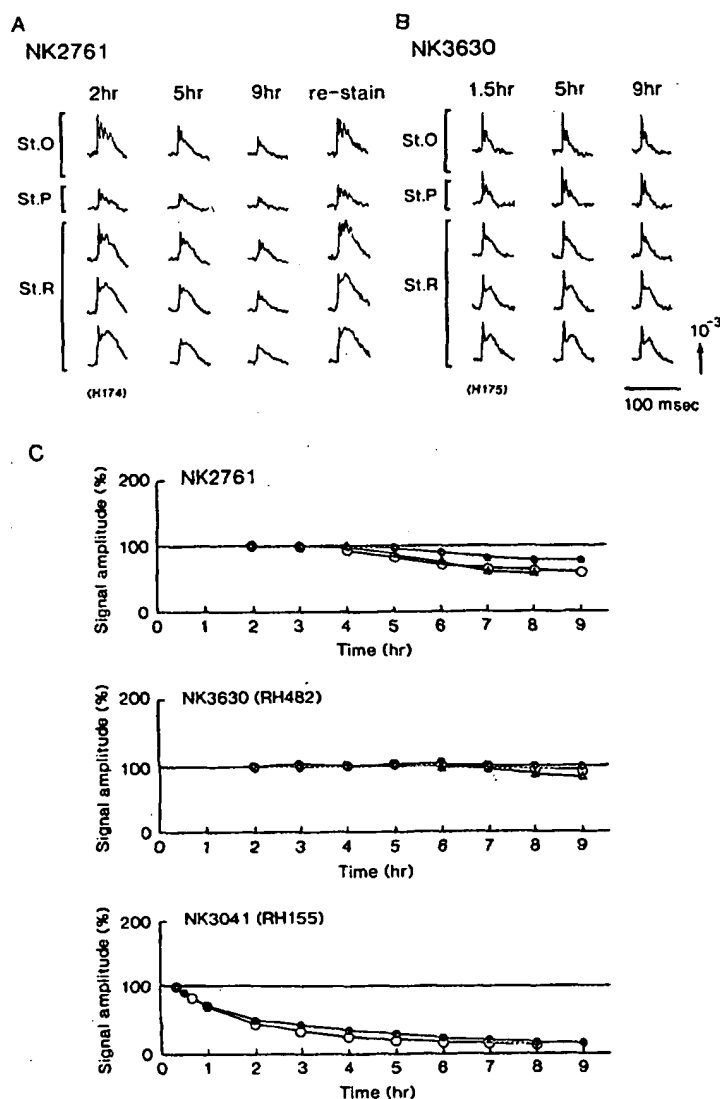


Fig. 6. Optical responses monitored for a long period. In *A*, recordings were made 2, 5 and 9 hr after the staining with NK2761, and in *B*, signals were obtained 1.5, 5 and 9 hr after the staining with NK3630. The dye concentration was 0.5 mg/ml. In *A*, the optical signals were restored after the preparation was restained with 0.5 mg/ml NK2761. (*C*) The amplitudes of the fast signals (mean of eight signals) detected from the stratum radiatum were plotted against the time from the staining (time 0). The optical signals were normalized with those at 2 hr for NK2761 (top) and NK3630 (middle), and at 20 min for NK3041 (bottom). The incident light was turned off except during the measuring period (about 5 sec per hr). Different symbols indicate different preparations ($n = 3$ for NK2761 and NK3630, $n = 2$ for NK3041).

was different from that of the merocyanine-rhodanine dyes: the null wavelength was around at 650 nm, and the maximum absorption changes were observed at 700 nm and 630 nm. These values are similar to those reported by Konnerth et al. (1987) in skate cerebellum. According to these experimental results, we used an incident wavelength of 700 nm in the following experiments.

TIME DEPENDENT CHANGE IN THE OPTICAL SIGNALS AFTER STAINING

For most dyes tested, the size of both the fast and slow optical signals was small just after the staining, and it gradually increased with time. Examples for NK3225 and NK3630 are presented in Fig. 4A and B. The size of the optical signals at 60 min was significantly larger than

at 10–20 min. This behavior was observed in every layer of the CA1 region. Figure 4C shows the time course of the slow-signal amplitude detected from the stratum radiatum stained with NK3630. The abscissa is the time after the staining, and the ordinate is the amplitude of the slow signals (baseline-to-peak) normalized to the size of the signals at 20 min. The maximal signal size was attained 60 min after the staining.

In the present experiment, the perfusion was stopped for 5 min during the staining. We checked the effects of this procedure in a preparation stained with NK3630. In Fig. 5, optical signals detected before stopping perfusion for 5 min (control), just after reperfusion (0 min) and 10 min after reperfusion (10 min) were compared. The fast and slow signals were slightly reduced with the cessation of perfusion, but they fully recovered after 10 min. Therefore, the suppression of the optical signals observed initially after staining is not due to ischemia.

Table 3. Effective recording time with no discernible change in the optical signals

Dye	Preparation reference	10% Reduction time	
		Fast signal	Slow signal
NK2761	H167	6 hr 13 min	6 hr 3 min
	H169	5 hr<	4 hr 20 min
	H174	4 hr 20 min	4 hr 12 min
	H187	4 hr 33 min	5 hr 8 min
NK2776	H195	9 hr<	9 hr<
	H197	8 hr 40 min	9 hr<
NK3224	H196	5 hr	4 hr 56 min
	H201	5 hr 24 min	5 hr 27 min
NK3225	H194	7 hr	6 hr 43 min
	H199	5 hr 24 min	6 hr
NK3630 (RH482)	H67	7 hr 20 min	7 hr 9 min
	H69	9 hr<	9 hr<
	H175	9 hr<	9 hr<
NK3041 (RH155)	H141	40 min	39 min
	H193	31 min	43 min
	H198	32 min	34 min

The time required for 10% reduction of the optical signal amplitude was evaluated for the fast and slow signals detected from the stratum radiatum. The values were measured from the plots shown in Fig. 6C.

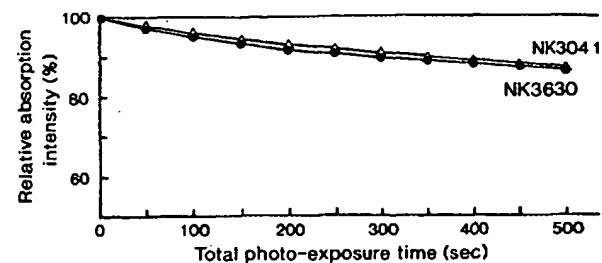
The time required for the maximum signal size depended on the dye. These results are summarized in Table 1. Of the dyes tested, NK3041 showed the fastest increase in signal size ($90\% < 10$ min), followed by NK3630 > NK2761 > NK3224 > NK3225, and then NK2776 ($90\% \sim 1.5$ – 2 hr). The rate of increase in signal was not changed by lowering the concentration of the dye, by increasing the perfusion rate, or by increasing the pre-incubation time (see NK2761 and NK3630 in Table 1).

SIGNAL SIZE AND SIGNAL-TO-NOISE RATIO

The signal size and the signal-to-noise ratio provide a good indication of which dyes are likely to be useful for monitoring transmembrane potential (Cohen et al., 1974; Ross et al., 1977; Gupta et al., 1981; for a review see Cohen & Salzberg, 1978). Thus, we examined the fractional change in transmitted light ($\Delta I/I$) and signal-to-noise ratio (S/N) for the best signals obtained from the preparations stained with the different dyes. In Table 2, the maximum sizes of $\Delta I/I$ and S/N measured in single sweep recordings are compared for the fast and slow signals.

When the merocyanine-rhodanine dyes were applied to the hippocampal slice, NK2761, NK3224 and NK3225 usually gave large signals and good signal-to-noise ratios: $\Delta I/I$ was 7.0 – 16.4×10^{-4} and S/N was 6.7 – 19.1 .

A



B

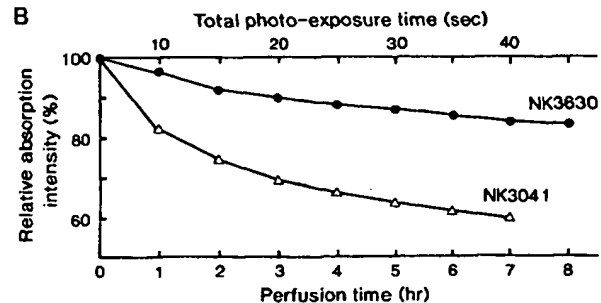


Fig. 7. (A) Effects of photo-illumination on the absorption intensity of the dye. The ordinate represents the relative absorption intensity (DC-background light intensity at time 0 divided by that at each time), and the abscissa is the time after the beginning of continuous illumination. (B) Effects of perfusion on the absorption intensity of the dye. The incident light was turned off except during the measuring period. The lower abscissa is the time after the staining (perfusion rate: 1 ml/min), and the upper abscissa is the total illumination time. Closed circles are for an experiment with NK3630 and open triangles are for an experiment with NK3041.

On the other hand, NK2776 provided smaller signals: $\Delta I/I$ was 4.4 – 10.8×10^{-4} and S/N was 3.5 – 10.0 . When we used NK2761 with a concentration of 0.2 mg/ml, the signal size and the signal-to-noise ratio were markedly smaller, suggesting that this concentration is not optimal.

Of the dyes tested, the oxonol dye, NK3041, gave the largest signals: $\Delta I/I$ was 11.0 – 35.0×10^{-4} . NK3630 also provided large signals. However, the signal-to-noise ratio of these oxonol dyes, especially of NK3630, was not as good as expected. The S/N were 8.6 – 17.6 for NK3041 and 2.9 – 9.3 for NK3630. In general, signal-to-noise ratio is proportional to the square root of the transmitted background light intensity, if the dominant noise is shot-noise (Waggoner & Grinvald, 1977; Salzberg, 1983; Grinvald et al., 1988). In the present experiment, the background light intensity of the oxonol dyes was much smaller than that of the merocyanine-rhodanine dyes under equal staining conditions and with equal illumination intensity (Table 2: I). Thus, the low transmitted light intensity seems to be the cause of the poor signal-to-noise ratio of the oxonol dyes.

EFFECTIVE RECORDING TIME

To evaluate the utility of the dyes for long-term continuous recording, we examined how long measurements can

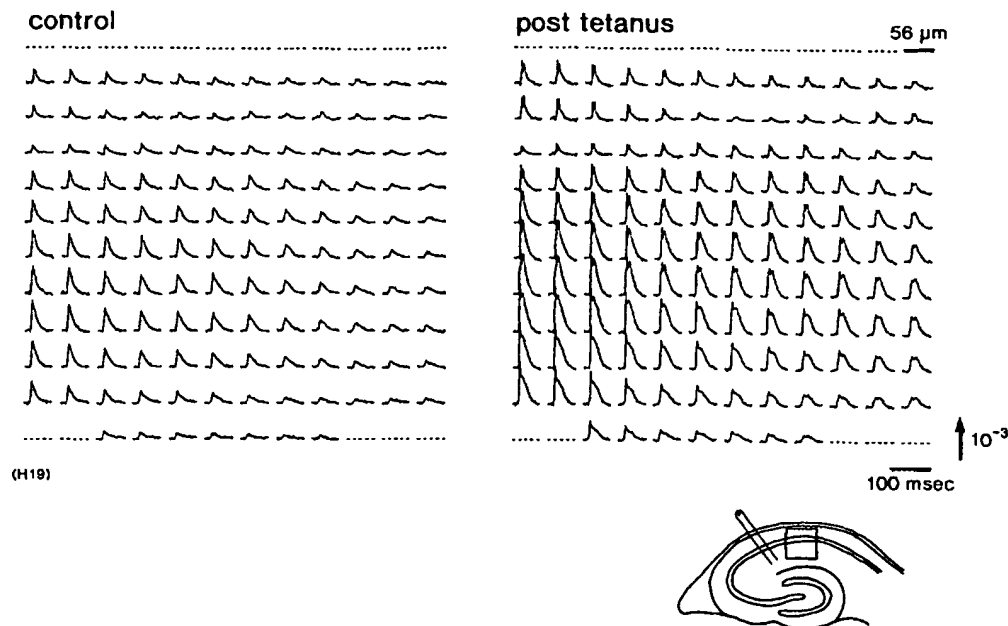


Fig. 8. Potentiation of the optical signals induced by tetanic stimulation (100 Hz/1 sec duration) delivered to the Schaffer collaterals. The preparation was stained with NK2761, and 8 trials were averaged.

be made with no discernible change in the optical responses. Figure 6A and B present optical signals monitored for 9 hr after the preparations were stained with NK2761 and NK3630. In this experiment, the incident light was turned off except during the measuring period (about 5 sec per hr). When we used NK2761 (Fig. 6A), the size of the fast and slow signals decreased gradually with time in every layer of the CA1 region. Both the signals were recovered in amplitude after the preparation was restained with NK2761, indicating that the deterioration of the optical signals is not due to decreased viability of the slice, but, rather, to lowered effectiveness of the dye. In the case of NK3630 (Fig. 6B), however, a significant reduction of the optical signals was not evident even after 9 hr.

In Fig. 6C, normalized signal amplitudes of the fast signals detected from the stratum radiatum are plotted against time, for several preparations stained with NK2761, NK3630 and NK3041. When we used NK2761 (Fig. 6C, top), the size of the optical signals was nearly constant for 4 hr, and then declined gradually. For NK3630 (Fig. 6C, middle), the optical signals were almost unchanged for 7 hr. On the other hand, when we applied NK3041 (Fig. 6C, bottom), the optical signal decreased rapidly, and the signal amplitude was reduced by 40% after 1 hr. Similar experiments were carried out using three other merocyanine-rhodanine dyes (NK3224, NK3225 and NK2776). The results are summarized in Table 3. Of the dyes tested, NK3041 exhibited the most rapid change in the optical signal size (10% reduction

<40 min), followed by NK2761, NK3224, NK3225, and then NK2776/NK3630 (10% reduction >9 hr).

Two possible mechanisms of the time-dependent change in the optical signals can be considered. One is photobleaching, which is caused by an exposure of the stained preparation to the illumination light. Another is a reduction of the amount of dye bound to the cell membranes, which is caused by perfusion or other experimental procedures. To identify the mechanism(s), we examined the effects of illumination and perfusion. The results are shown in Fig. 7A and B, respectively. In Fig. 7A, the time course of photobleaching is compared for two oxonol dyes, NK3630 and NK3041. The abscissa is the illumination time, and the ordinate is the normalized background light intensity monitored without electrical stimulation under continuous illumination. Between NK3630 and NK3041, no significant difference was observed in the rate of increase in the transmitted light intensity (a decrease in dye absorption). In Fig. 7B, the effect of perfusion is compared for NK3630 and NK3041. In this experiment, the incident light was turned off except during the measuring period (about 5 sec per hr). The normalized transmitted light intensity is plotted against the time of perfusion (1 ml/min; lower abscissa), together with the time of total illumination (upper abscissa). In Fig. 7B, the rate of increase in the transmitted light intensity (a decrease in absorption) was more rapid in NK3041 than in NK3630. We suggest that this difference is due to a difference in a dissociation rate of the dye from the cell membrane, and this is the cause

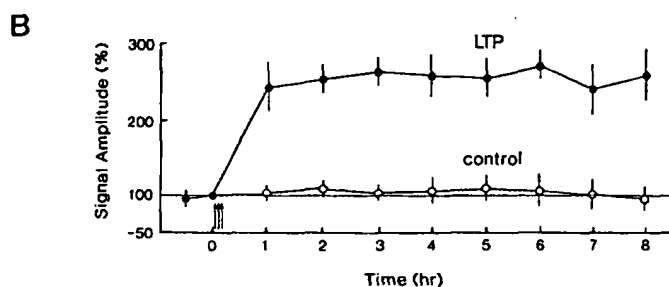
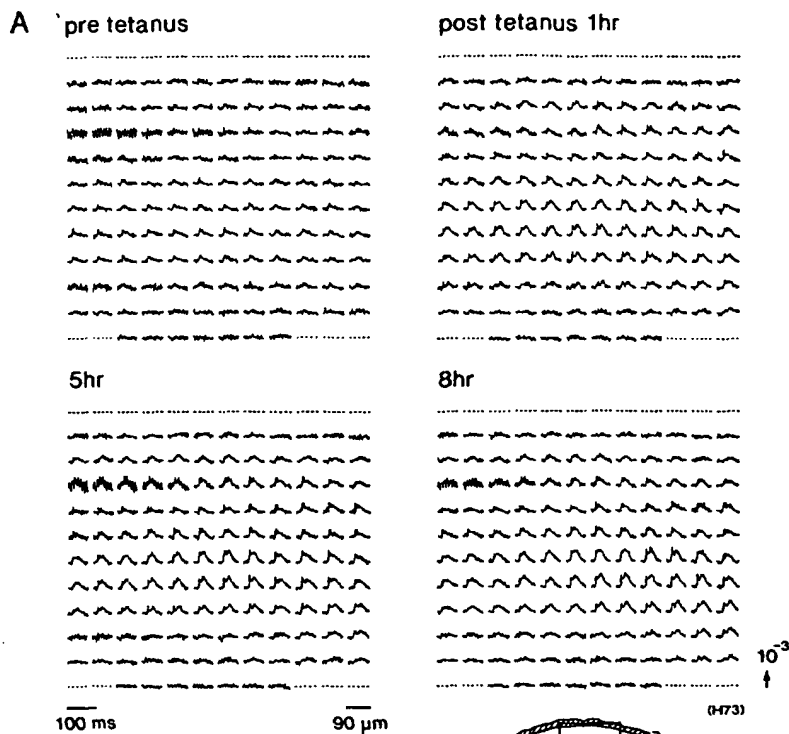


Fig. 9. (A) Long-term recording of the potentiation of the optical signals induced by tetanic stimulation (400 Hz/25 msec duration \times 5 trains \times 10 bursts: see Methods). The preparation was stained with NK3630, and two trials were averaged. (B) Normalized signal amplitudes of the EPSP-related slow optical signals detected from the stratum radiatum. Mean \pm SD ($n = 8$ positions) of the signal amplitude is plotted against the time. Closed and open circles are the data obtained with and without tetanization, respectively. Arrows indicate tetanization.

of the difference in the time course of the optical signal change observed in Fig. 6 and Table 3.

APPLICATION TO MONITORING LTP

Based on the experimental results represented above, we applied the multiple-site optical recording method to the hippocampal slice to monitor long-term potentiation. Figure 8 shows a typical example of the potentiation of the optical signals induced by tetanic stimulation. The preparation was stained with NK2761 and the tetanus was delivered to the Schaffer collateral pathways. Tetanization increased the amplitude of the EPSP-related slow optical signals. In addition, the initial spike portion

seems enhanced, and in some regions a second spike appeared. The change was most evident in the region of the stratum radiatum. Thus, in the following experiment, we focused on the EPSP-related signals evoked in this region.

Figure 9 shows a long-term recording of optical signals made for 8 hr after tetanization. In this experiment, we used NK3630, and the recordings were made only twice every hour (average of two trials in each recording) to minimize the effects of photobleaching. Although the signal-to-noise ratio of the original recording was not large, it is clearly demonstrated that the slow signal amplitude was increased significantly by the tetanus, and that this potentiation lasted for 8 hr. In Fig. 9B, the time course of the slow signal amplitude, normalized at time 0 (just before tetanization), is presented. From these data, it is demonstrated that the optical recording tech-

nique with a voltage-sensitive dye can be used effectively for the study of LTP in in vitro slice preparations.

Discussion

In the present experiments, we screened several voltage-sensitive dyes with an emphasis on absorption in the hippocampal slice preparation. Screening of dyes in a new preparation seems to be crucial for a successful application of the optical technique, because it has been shown that the sensitivity (the signal size and the signal-to-noise ratio), wavelength dependence, and other characteristics of the dyes differ from species to species and from preparation to preparation (Ross & Reichardt, 1979; Senseman & Salzberg, 1980; Grinvald et al., 1988). Indeed, an oxonol dye, NK3041 (RH155), provided large optical signals in the hippocampal slice, although it gave very small signals in the embryonic nervous systems (Momose-Sato et al., 1995).

The ideal voltage-sensitive dye is sensitive to changes in transmembrane potential and has little or no pharmacological and/or phototoxic actions. In addition, it is required that bleaching of the dye is small. The present results demonstrate that useful absorption probes of membrane potential are available from among the merocyanine-rhodanines and the oxonols. In the present experiment, large signal-to-noise ratios were obtained for NK2761, NK3224, NK3225 and NK3041 (RH155). Although NK3041 showed the largest signal and the fastest increase in the signal size after staining, this dye seems not ideal for monitoring neural responses in the hippocampal slice. First, NK3041 often exhibited a second slow component with long duration, which was not observed with other dyes. Second, the reduction of the optical signals due to dye washout was so rapid that a stable recording could not be performed. The shape and the size of the optical signals using NK2761, NK3224 and NK3225 were nearly constant for 4 to 6 hr, so these dyes appear to be useful for a short-term recording in the hippocampal preparations. The signal-to-noise ratio of NK3630 (RH482) was slightly smaller than that of the merocyanine-rhodanine dyes. However, this dye permitted optical measurements with no discernible change in the signal size over periods of 8 hr or longer. Thus, for long-term continuous recording, NK3630 seems best in the present experiment. The decrease in the optical signals is likely to be due to a dissociation of the dye molecule from the cell membrane caused by perfusion. Therefore, if these dyes are applied to a preparation that does not require perfusion (e.g., cultured slices), even longer recordings might be possible.

When we applied the merocyanine-rhodanine dyes and the oxonol dye NK3630, the size of the fast and slow signals was usually small just after the staining, and it gradually increased with time. This phenomenon has not

been observed either in embryonic nervous systems or in cardiac tissues (Kamino, 1990, 1991; Momose-Sato et al., 1995), suggesting that the interaction between the dye and the cell membrane is a complex one in a variety of preparations.

LONG-TERM POTENTIATION

The optical recording technique has been applied to the hippocampal slice preparation for short-term recording of LTP (Saggau et al., 1986) and epileptiform potentials (Albowitz & Kuhnt, 1991). In the present experiment, we have succeeded in monitoring LTP for at least 8 hr. As is the case with behavioral memory, LTP in the hippocampal CA1 region and in the dentate gyrus consists of different stages: late LTP, lasting longer than 4 hr, can be distinguished from early LTP, lasting minutes or several hours, using inhibitors of protein synthesis (Frey et al., 1988; Otani et al., 1989; Frey & Morris, 1997). LTP is also classified into three phases, viz., LTP1, LTP2 and LTP3, according to the time constants of their decay (Abraham & Otani, 1991; Abraham et al., 1993). It has been suggested that the late phase of LTP is dependent on *de novo* synthesis of mRNA. However, the experimental effects of an RNA synthesis blocker, actinomycin D, are still confusing (Otani et al., 1989; Nguyen et al., 1994; Nguyen & Kandel, 1996; Frey et al., 1996). We are now investigating the effects of some inhibitors of protein synthesis on LTP, using the optical recording technique, and the voltage-sensitive dyes that have proven to be useful in the present experiment.

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